PLANT PARASITIC ORGANISMS IN THE RIZOSPHERE OF APPLE TREES IN THE WESTERN CAPE, WITH SPECIAL REFERENCE TO WOOLLY APPLE APHID

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entire or in part, been submitted at any university for a degree.

ABSTRACT

Various aspects of the biology and ecology of woolly apple aphid, *Eriosoma lanigerum*, were investigated, including initial galling damage caused by *E. lanigerum* to the roots of apple trees, the possible relationship between *E. lanigerum* and *Xiphinema* and *Pratylenchus* nematodes and the effectiveness of Biostart 2000® and Furfural® as possible control agents of *E. lanigerum* in the orchard.

Preliminary root damage by first instar *E. lanigerum* feeding was characterized by the mechanical injury of endodermis and parenchyma tissues. Damage by second, third and fourth instar *E. lanigerum* was similar, but the symptoms were more pronounced. Damage caused by adults included a pronounced swelling at infected areas of the root. Cell walls hardened until the root was radially strengthened with sclerenchyma tissue and non-conducting xylem vessels while the cuticle expanded greatly through the growth of cork-like cambium tissue.

There was no direct relationship between the population dynamics of *E. lanigerum* and those of *Xiphinema* and *Pratylenchus* nematodes. The occurrence of *E. lanigerum* appeared to be seasonal while *P. penetrans* and *Xiphinema* numbers fluctuated erratically. Undamaged root nitrogen levels seemed to correspond with the normal root growth cycle. Nitrogen levels from galled roots were significantly lower than those of undamaged roots, probably due to *E. lanigerum* feeding. Soils rich in fine sand and clay sustained higher populations of *E. lanigerum* and *Xiphinema* than sandy soils. The number of *E. lanigerum* found in soil samples correlated well with the damage index allocated to the samples. The numbers of *Xiphinema* found in soil samples also correlated well with the damage index allocated to the samples according to suspected *Xiphinema* damage symptoms.

Both Biostart 2000® and Furfural® were effective as control agents of woolly apple aphid. Furfural®, a chemical waste product of the sugarcane industry, was however not as effective as Biostart 2000®, a product that includes an activator and three bacterial species, *Bacillus laterosporus*, *B. chitinosporus* and *B. licheniformis*. The bacteria in the Biostart 2000® treated pots could replicate themselves under suitable conditions while Furfural® dilutes with each watering. Biostart 2000® is also easier to prepare than Furfural® since the components of Biostart 2000® readily mix to form a paste easily thinned by water, whereas Furfural® is an oily substance that does not easily disperse in water.

Root damage was initiated soon after *E. lanigerum* started feeding, however there was no apparent relationship between *E. lanigerum* and the nematode species. The most promising, environmentally friendly control measure was Biostart 2000®.

OPSOMMING

Verskeie aspekte van biologie en die ekologie van die appel bloedluis, *Eriosoma lanigerum,* was ondersoek insluitende aanvanklike galvorming veroorsaak deur *E. lanigerum* op wortels van appelbome, die moontlike verwantskap tussen *E. lanigerum* en *Xiphinema* en *Pratylenchus* nematodes en die effektiwiteit van Biostart 2000® en Furfural® as moontlike beheeragente van *E. lanigerum* in die boord.

Aanvanklike wortelskade deur eerste instar *E. lanigerum* voeding was gekenmerk deur die meganiese beskadiging van endodermale en parenchiem weefsel. Skade veroorsaak deur tweede, derde en vierde instar *E. lanigerum* was soortgelyk alhoewel die simptome meer beklemtoond was. Skade deur volwassenes het 'n meer duidelike swelsel by geïnfekteerde wortelareas ingesluit. Selwande het verhard totdat die wortel radiaalsgewys versterk was met sklerenchiem weefsel en nie-geleidende xileemvate terwyl die kutikula grootliks toegeneem het deur die groei van kurkagtige kambiumweefsel.

Daar was geen direkte verwantskap tussen die bevolkingsdinamika van *E. lanigerum* en dié van *Xiphinema* en *Pratylenchus* nematodes nie. Die voorkoms van *E. lanigerum* was seisoenaal terwyl *P. penetrans* en *Xiphinema* se getalle onvoorspelbaar gefluktueer het. Onbeskadigde wortel stikstofvlakke het ooreengestem met die normale wortel groeisiklus. Stikstof vlakke van galwortels was noemenswaardig laer as dié van onbeskadigde wortels, heel waarskynlik as gevolg van voeding deur *E. lanigerum*. Grond ryk aan fyn sand en klei het groter bevolkings van *E. lanigerum* en *Xiphinema* onderhou as sanderige gronde. Die aantal *E. lanigerum* in grondmonsters het goed ooreengestem met die skade indeks wat aan die monsters toegeken was. Die aantal *Xiphinema* in grondmonsters het ook goed ooreengestem met die beskadigingsindeks wat aan die monsters toegeken is weens vermoedelike *Xiphinema* skade simptome.

Beide Biostart 2000® en Furfural® was effektief as beheeragente van die appelbloedluis. Furfural®, 'n afvalproduk van die suikerriet industrie, was egter minder effektief as Biostart 2000®, 'n produk bestaande uit 'n aktiveerder en drie bakterie spesies, *Bacillus laterosporus*, *B. chitinosporus* en *B. licheniformis*. Die bakterië in die Biostart 2000® behandelde potte kon vermeerder onder gunstige toestande terwyl Furfural® na elke besproeiing verdun het. Biostart 2000® is ook makliker om aan te maak as Furfural® aangesien die bestanddele van Biostart 2000® geredelik meng tot 'n wateroplosbare pasta, terwyl Furfural® 'n olierige vloeistof is wat moeilik 'n waterige suspensie vorm.

Wortelskade het plaasgevind kort nadat *E. lanigerum* begin voed het, alhoewel daar geen duidelike verwantskap tussen *E. lanigerum* en nematode spesies voorgekom het nie. Die mees belowende omgewingsvriendelike beheermaatreël was Biostart 2000®.

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CONTENTS

CHAPTER 1
INTRODUCTION

Eriosoma lanigerum (Hausmann) (Homoptera: Aphididae), or woolly apple aphid, is native to North America where it feeds naturally on white elm, *Ulmus americanus*, a tree exotic to South Africa. The present distribution of *E. lanigerum* is limited to between latitudes 64° N and 57° S and longitudes 185° E and 159° 75' W (Asante 1994). It was first described by Friedrich Hausmann in 1802 (Baker 1915) and has been reported as a pest in commercial orchards and nurseries throughout the world (Walker *et al.* 1988). Woolly apple aphid has also been known as a pest of apple trees, *Malus domestica* Borkh, for the last 200 years (Hoyt & Madson 1960). Feeding takes place on shoots, branches, in pruning and other wounds, leaf axils and on the roots of apple trees.

In the Northern Hemisphere the aphids overwinter in the egg stage before passing one or two early spring generations on elm trees. Early summer winged forms migrate to apple trees, hawthorn and related plants in the Rosaceae where they settle and reproduce sexually, producing eggs that overwinter. In late summer some migrate down to the tree roots where their feeding produces gall-like excrescences. They multiply before migrating up into the aerial parts the next spring (Annecke & Moran 1982).

Both winged and apterous females occur in South Africa (Annecke & Moran 1982). Their bodies are covered with white waxy filaments, and they both produce live young. Woody galls on apple roots resulting from aphid feeding are formed by cell hypertrophy and hyperplasia, replacing normal xylem tissue and hampering root transport of water and nutrients (Brown *et al.* 1991). Subterranean aphid populations reduced potted tree growth more than aerial aphid populations did (Weber & Brown

1988). Root damage caused by *E. lanigerum* is, however, more difficult to document and quantify due to the difficulty of sampling subterranean aphids (Damavandian 2000).

E. lanigerum was first recorded in South Africa in 1895 and was distributed throughout the whole country, being the worst of all apple pests (Annecke & Moran 1982). In the past control of *E. lanigerum* was based solely on chemical sprays and resistant rootstocks. Fuller (1904), Staniland (1924), Knight *et al.* (1962), Rock & Zeiger (1974), Taylor (1981) and Young *et al.* (1982) have reported on apple rootstocks resistant to *E. lanigerum.* Myburgh (1962) suggested the use of resistant rootstocks as the only permanent solution against *E. lanigerum* infestation. Blommaert *et al.* (1968) recommended that Merton 793 rootstocks should be used instead of seedling rootstocks. A strain of *E. lanigerum*, which can tolerate the resistance factor in Northern Spy and related rootstocks, has however evolved in some areas of South Africa (Giliomee *et al.* 1968).

Early methods of controlling subterranean woolly apple aphid included the scraping away of the surface soil before nearly boiling water was poured around the base of the tree, saturating the soil several inches deep (Fuller 1904). Tobacco dust was also applied after the surface soil was removed, sprinkling it around the tree base up to a distance of two or three feet.

Blommaert *et al.* (1968) reported that phorate fluid was very promising in preliminary trials while Myburgh & Van Niekerk (1964) found that phorate had a remarkable debilitating effect on subterranean *E. lanigerum* populations. Tarr & Hyde-Wyatt (1965) proposed the use of vamidothion, a systemic insecticide, for the control of aerial aphid populations. Vamidothion was eventually widely used for the control of woolly apple aphid (Loubser 1968). Swart *et al.* (1992) reported its use in a tank mixture in combination with chlorpyrifos. Pringle *et al.* (1994) have since

reported tolerance to vamidothion in the Elgin area of the Western Cape, South Africa.

Subterranean populations of *E. lanigerum* were controlled for at least three successive seasons by a single application of imidacloprid to the soil (Pringle 1998). Aerial infestations were greatly reduced following the adequate control of root infesting populations.

Plant parasitic nematodes are found in the soil in apple orchards together with *E. lanigerum*, especially dagger nematodes, *Xiphinema* (Cobb) (Dorylaimida: Longidoridae) and lesion nematodes *Pratylenchus* (Filipjev) (Tylenchida: Pratylenchidae). Nematodes deplete reserves of plants and reduce resistance to drought, stress and diseases by interfering with the metabolic activity of the plants (Griffin *et al.* 1996).

Dagger nematodes are extremely common in South Africa, occuring in all climatic regions and soil types. They are medium-sized to large, up to 10 mm or more in length, and are armed with a long spear (Heyns 1971). Halbrendt & Brown (1993) demonstrated that *Xiphinema* species pass through three or four juvenile stages before becoming adults. *X. americanum* completes one generation per year (Griffin *et al.* 1996).

X. americanum is a well-known ectoparasitic virus vector in South Africa and is morphologically closely related to *X. brevicolle*, both frequently occurring together in orchard soils (Heyns 1971). Kotcon *et al.* (1991) describes dagger and lesion nematodes as the dominant nematode pests of apple orchards in the northeastern United States. *X. americanum* is a well known and economically important vector of nepoviruses notably tomato ringspot virus (TmRSV) and tobacco ringspot virus (TbRSV) (Halbrendt & Jing 1995). TmRSV causes, amongst others, tree mortality due to union necrosis in certain rootstock/scion combinations as well as the lethal

stem pitting disease (Halbrendt 1993). Liskova & Brown (2000) describe several *Xiphinema* spp. as vectors of cherry leaf roll nepovirus to walnut (*Juglans regia* L.). In addition Pruyne *et al.* (1994) showed that *Xiphinema* spp. and *Pratylenchus penetrans* caused severe stunting of apple seedlings. Six species of *Xiphinema*, including *X. brevicolle* and *X. americanum* and *P. penetrans* caused gradual dying and weakening of fruit trees, especially apricot, in Bulgaria (Ivanova *et al.* 1997).

Pratylenchus spp. are small nematodes, up to 0.4 mm in length, armed with a short massive spear with spear knobs (Heyns 1971). They pass through several generations annually (Griffin *et al.* 1996). Lesion nematodes are all obligate plant parasites, living as endoparasites in underground plant tissues. *P. penetrans* has been recorded on more than 350 hosts and is widely distributed throughout the temperate areas of the world (Mizukubo & Adachi 1997). Hosts include various field crops such as tobacco, fruit and forest trees, vegetables such as potatoes, shrubs and ornamentals.

Feeding by *Pratylenchus* nematodes results in necrotic lesions on the roots and often occur in numbers large enough to cause economic damage (Heyns 1971). Lesion nematodes cause root damage and promote root rot (Halbrendt 1993, Pinochet 1997, Pinochet *et al.* 1995) in association with several species of fungi, including *Pythium*, *Fusarium* and *Phytophthera* (Utkhede *et al.* 1992). *Pratylenchus* spp. and pathogens are directly related to apple replant disease (ARD) (Dullahide *et al.* 1994, Stirling *et al.* 1995). Pinochet *et al.* (1994) found various isolates of *P. vulnus* to be especially damaging to *Malus silvestris* and M26 apple rootstocks.

Pratylenchus and *Xiphinema* nematodes are controlled by various methods including soil fumigation (Halbrendt 1993), fallowing, solarisation, green manuring, organic amendments, non-volatile nematicides (Stirling *et al.* 1995) and nematicides such as carbofuran and tenekil (Khan *et al.* 1996).

The possibility of mutualism between *E. lanigerum* and dagger and lesion nematodes, although obvious, has not been investigated as yet. Therefore, an investigation of the interaction of woolly apple aphid with dagger and lesion nematodes on the roots of apple trees was considered necessary. This would provide a better understanding of these subterranean pests as well as a basis for a possible integrated approach to the control of edaphic *E. lanigerum*, *Xiphinema* and *Pratylenchus* nematodes. Although gall damage caused by *E. lanigerum* has been documented, symptoms of early gall damage remain lacking. Early feeding damage symptoms by the various nymphal stages of *E. lanigerum* were therefore investigated. The future of pest management will lean more towards biological control. Therefore, a bacterial preparation, Biostart® 2000, was tested for its effects on *E. lanigerum*. Furfural, a byproduct from the sugarcane industry, is being used for the control of nematodes. This was also compared for its effects on *E. lanigerum*.

The research project therefore focused specifically on the following:

1. Identification of the development of root damage caused by each instar of *E. lanigerum*;
2. The study of the possible interactions between subterranean *E. lanigerum* and nematodes, specifically *Xiphinema* and *Pratylenchus;* and
3. The possible use of Biostart® 2000 and Furfural as control agents against *E. lanigerum.*

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CHAPTER 2

DEVELOPMENT OF GALL DAMAGE ON APPLE ROOTS BY WOOLLY APPLE APHID

Subterranean woolly apple aphid, *Eriosoma lanigerum* (Hausmann), attacks apple tree roots, causing galls and restricting water and nutrient flow (Brown *et al.* 1991). This leads to reduced trunk and foliage growth (Brown & Schmitt 1990) and reduced yield. Root galls began forming on potted M7 apple roots after only 4 – 8 weeks, disrupting the nutrient balance and reducing the tissue weight of infected roots (Brown *et al.* 1995). Foliar nitrogen and phosphorus levels were also reduced (Weber & Brown 1988). As an orchard matured, more trees became infested with *E. lanigerum* (Brown 1986).

Although many aspects regarding the reaction of the host plant to *E. lanigerum* feeding are well documented, no information is available on the damage caused by *E. lanigerum* during the initial stages of infestation. Information on the histology and morphology of galls caused by *E. lanigerum* is available (Brown *et al.* 1991), but this does not include the initial stages of damage resulting from *E. lanigerum* feeding. Therefore, the objective of the present study was to examine and record the sequence of symptoms as they occur from the onset of feeding by *E. lanigerum* on the roots of potted apple trees.

2.1 MATERIAL AND METHODS

2.1.1 Tree material and project conditions

The apple trees (*Malus domestica*) used were cultivar Royal Gala budded onto M7 rootstock. This rootstock is susceptible to *E. lanigerum* (Crane *et al.* 1936, Rock & Zeiger 1974, Sen Gupta & Miles 1975). The 20 one-year-old trees were kept in a cold store at 4 °C for three months to satisfy their cold requirements, after which they

were planted in 10 pots in commercial sterile potting soil on the 16th of August 2000. The project was conducted under laboratory conditions at a constant temperature of 25 °C and 12 hours lighting each day. The soil was kept moist by frequent watering. The potted trees were allowed to sprout and the roots were infested with first instar *E. lanigerum* crawlers on the 30th of August 2000.

2.1.2 Collection and treatment of aphids

The crawlers were obtained as progeny of aphids collected from apple tree roots from a commercial orchard at Oak Valley Farm in the Grabouw region of South Africa (34°9'S, 19°3'15"E). Adults were carefully removed from the roots with a small brush and placed on filter paper in petri dishes. The petri dishes were placed in an incubator at 23 °C and 80 % relative humidity (RH) in the dark. After 24 hours the crawlers were removed. Each of the 20 trees was infected with 50 crawlers that were placed on newly exposed roots. The infected areas were covered with pieces of filter paper, increasing the protection of crawlers against desiccation and light. The surface of each pot was covered with a circle of hessian wrapped around the trunk of the tree to provide dark conditions to allow crawlers to settle. Crawlers were observed daily to ensure adequate infestation.

2.1.3 Sampling of roots

The average time for woolly aphid development from crawler to adult at 25 °C is approximately 14 days with development from the first to the second instar taking longer than the other stages (Damavandian 2000). Roots were inspected daily for signs of moulting by the aphids. The aphids settled by the 4th of September 2000, four days after infestation, and the first batch of roots was collected on the same day. Most crawlers had completed their first moult by the 7th of September 2000 when the

second batch of roots was sampled. The third, fourth and fifth samples were inspected on the 11th, 14th and 18th of September 2000 respectively. These coincided with the second, third and fourth moults (Damavandian 2000). Root samples shorter than 50 mm in length with a diameter of 5 mm or less were taken from areas were woolly apple aphid feeding had been taking place. Roots were removed using a sterilized scalpel blade and placed in a solution of fluoroacetic acid (FAA).

The roots were soaked in the FAA solution for at least 48 hours before cross-sections of 20 µ were made using a Leica CM 1100 cryostat microtome at – 20 °C. Cross-sections from undamaged roots were made for comparison with roots on which aphids had fed. These undamaged roots were taken from the same pots as the samples upon which *E. lanigerum* had been feeding. Care was taken to disturb roots as little as possible during sampling. The cross-sections from undamaged roots were cut to a thickness of 10 µ, since these roots were not damaged by aphid feeding and did not tear as easily as was the case with the damaged roots. Sections were placed on slides and fixed in lactic acid before being sealed under cover slips, using glyceal, prior to being photographed. Lactic acid was used, since it is a clear liquid (unlike lactophenol) that does not evaporate rapidly.

2.2 RESULTS

Undamaged vascular tissue, endodermis, cortex parenchyma, epidermis and cuticle can be seen in Fig. 2.2.1. Damage to the parenchyma tissue of the cortex (E, Figs 2.2.2 to 2.2.6) was caused by the feeding of all instars of *E. lanigerum* as the aphid proboscis probed through cells, removing cell contents and killing the cells.

During feeding by the first instar, the epidermal tissue (F, Fig. 2.2.2) and parenchyma tissue (E, Fig. 2.2.2) of the cortex on the side of the entry wound were destroyed, leaving a V-shaped intrusion. This extended to the vascular cylinder

surrounded by the endodermis (D, Fig. 2.2.2). Damage to the parenchyma tissue of the cortex can be seen (E, Fig. 2.2.2), especially when compared to the undamaged root tissues (Fig. 2.2.1). Parenchyma cell walls (E, Fig. 2.2.2) were broken in some places, leading to increased intercellular air spaces.

Second instar *E. lanigerum* damage to the parenchyma tissue of the cortex was extensive (E, Fig. 2.2.3). Damage was more extensive on the side of the entry wound (J, Figs. 2.2.2 to 2.2.6).

Feeding by the first, second and third crawlers did not damage the vascular bundles (Figs 2.2.2 to 2.2.4). However, large amounts of the cortex parenchyma were removed (E, Figs 2.2.2 to 2.2.4), even from areas not adjacent to where the proboscis entered, suggesting that damage was not only caused by the physical penetration of the stylets, but also by the chemical action of the saliva. Damage to the endodermis (D, Fig. 2.2.5) was apparent after feeding by 4th instar *E. lanigerum*. Damage to the vascular bundle only appeared (Figs 2.2.6, 2.2.7) during feeding by adult *E. lanigerum*.

Damage to the growing root tissues resulting from third instar aphid feeding caused the root to split lengthwise on the side damaged by proboscis entry (I and J, Fig. 2.2.4). The split stretched from the epidermis to the endodermis of the vascular bundle (D, Fig 2.2.4). The vascular bundle was mostly undamaged as it typically contains xylem bundles. Aphids primarily feed on parenchyma cell and phloem bundle contents (Klingauf 1987). Apart from the lengthwise split, damage caused by third instar aphid feeding was similar to that caused by first and second instar feeding.

Root tissue began to visibly respond to fourth instar *E. lanigerum* feeding. Relatively large intercellular spaces were found between the parenchyma cells of the cortex (E, Fig. 2.2.5) on the side of the entry wound. Outwardly there was a slight swelling of the root at infected areas. This suggested the involvement of enzymatic

damage, as purely mechanical damage would only have resulted in tears and broken cells.

The swelling of the infected area of the root had increased markedly by the time the woolly apple aphids moulted into their fifth and final instar (Fig. 2.2.6). The process of cell wall hardening continued until the whole root was radially strengthened with reams of dead sclerenchyma cells in vascular rays and non-conducting xylem vessels (K, Fig 2.2.6) while the cuticle had grown to many times its original thickness through the growth of cork-like cambium tissues (G, Fig. 2.2.6). Although samples were not taken for comparison from undamaged roots of a similar age, the infected area was more swollen than the normal areas surrounding it. Xylem and phloem formation was abnormal as a result of the tears through the vascular rays and disjointed occurrence of secondary (non-conducting) xylem and phloem tissues (K, Fig. 2.2.6). Growing root tissue caused a lengthwise split both internally and externally, coinciding with the entry point of the aphid stylets (J, Figs. 2.2.2, 2.2.4 and 2.2.6).

The stylets of two adult woolly apple aphids are visible along with the tear made by their feeding (Y, Fig. 2.2.7). Phloem columns (X, Fig. 2.2.7) were broken, hampering the flow of nutrients. The space created by the aphid stylets (W, Fig. 2.2.7) was filled with the contents of destroyed cells.

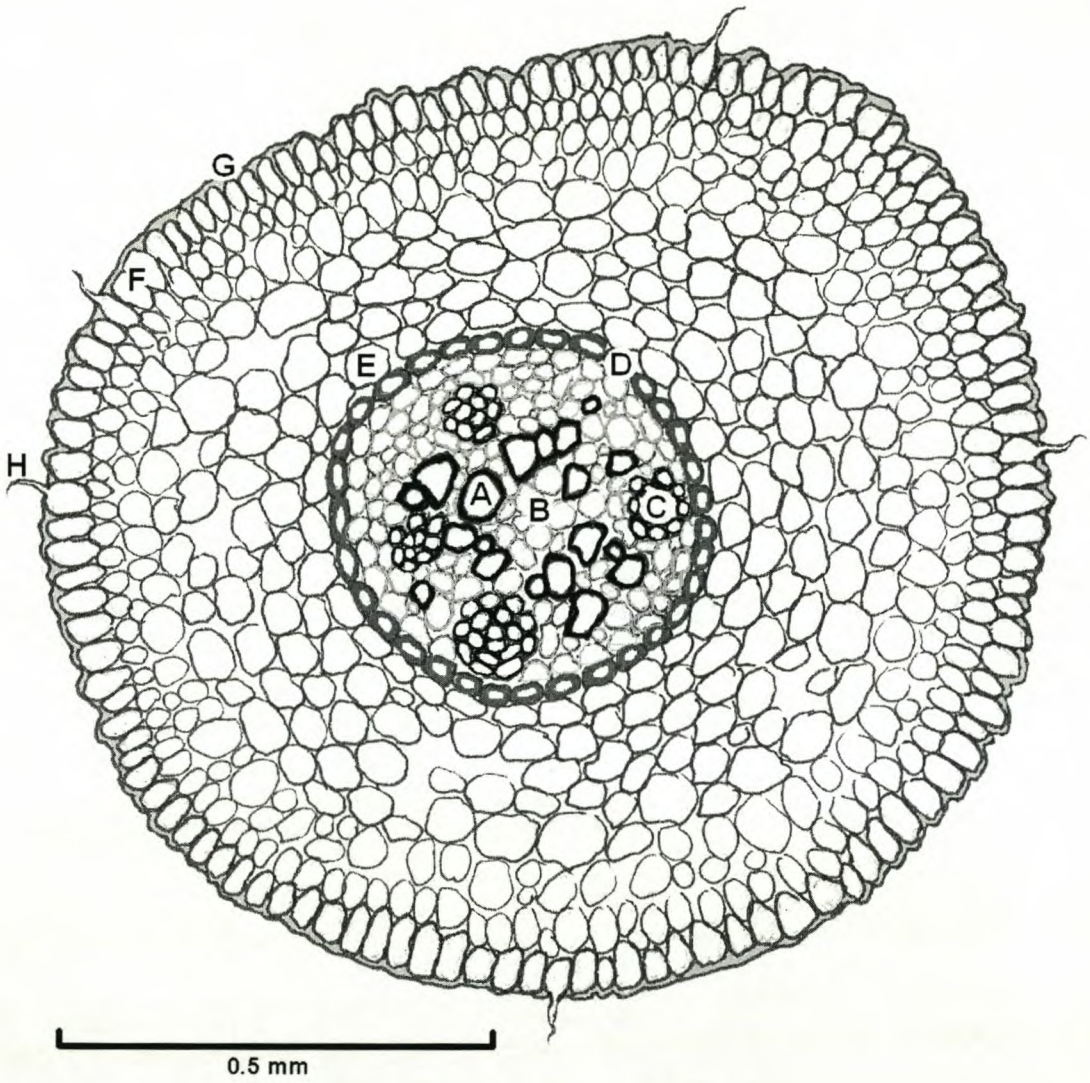

Fig. 2.2.1: Section cut from an undamaged apple root. (A) Primary xylem vessel. (B) Parenchyma tissue of the stele. (C) Primary phloem elements. (D) Endodermis. (E) Parenchyma tissue of the cortex. (F) Epidermis. (G) Cuticle. (H) Trichome.

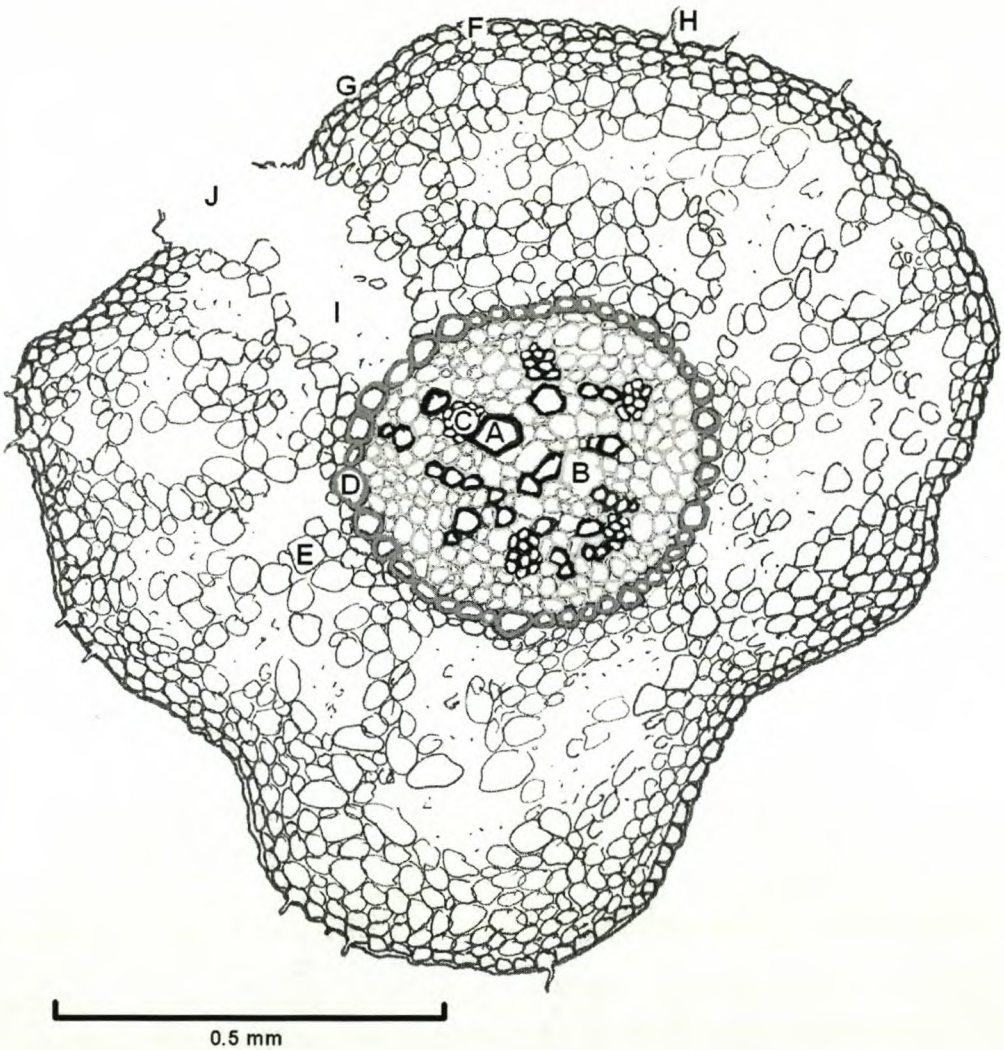

Fig 2.2.2: Section cut from an apple root on which a first instar *Eriosoma lanigerum* had been feeding. (A) Xylem vessel. (B) Parenchyma tissue of the stele. (C) Phloem elements. (D) Endodermis. (E) Parenchyma tissue of the cortex. (F) Epidermis. (G) Cuticle. (H) Trichome. (I) Area of damage caused by *E. lanigerum* feeding. (J) Entry point of stylet.

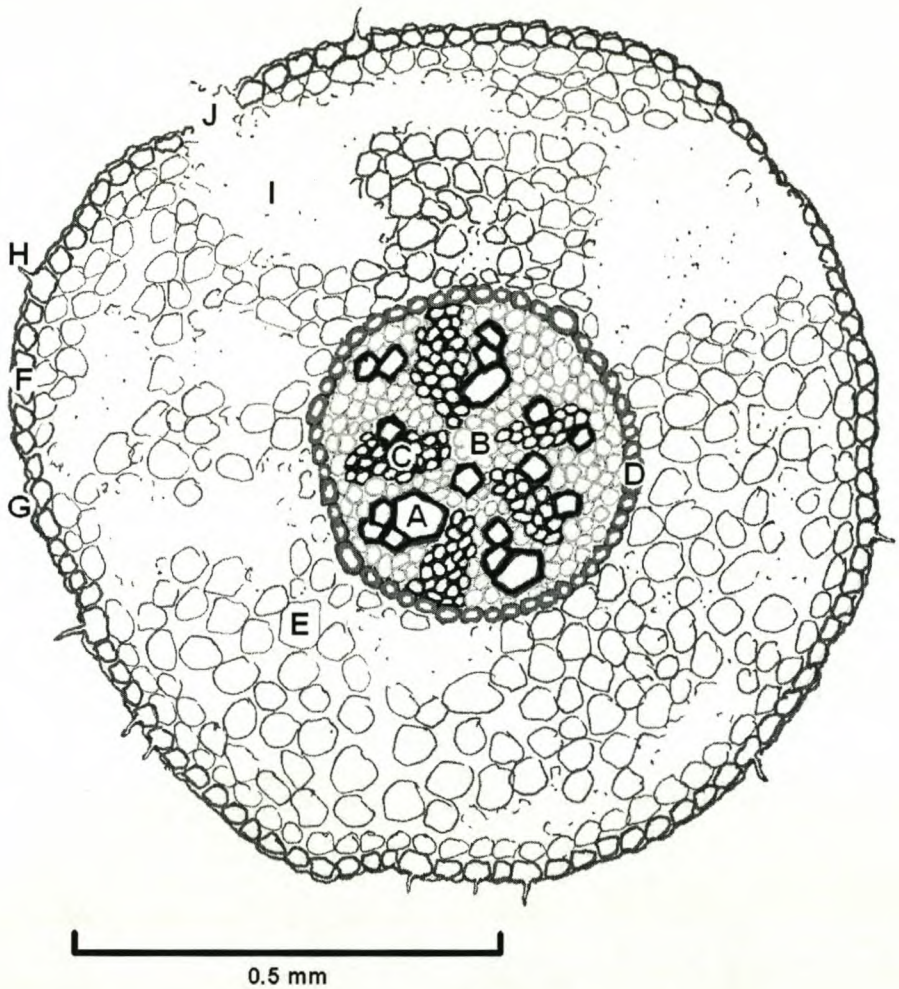

Fig. 2.2.3: Section cut from an apple root on which a second instar *Eriosoma lanigerum* had been feeding. (A) Xylem vessel. (B) Parenchyma tissue of the stele. (C) Phloem elements. (D) Endodermis. (E) Parenchyma tissue of the cortex. (F) Epidermis. (G) Cuticle. (H) Trichome. (I) Area of damage caused by *E. lanigerum* feeding. (J) Entry point of stylet.

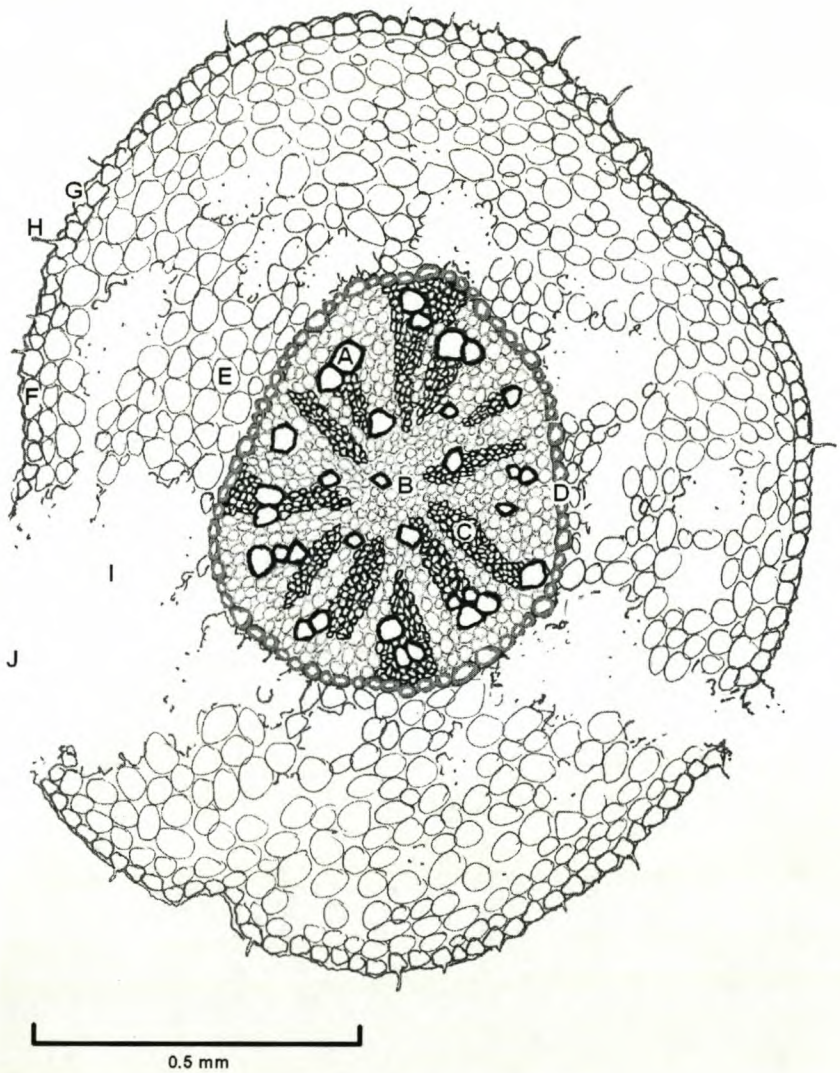

Fig. 2.2.4: Section cut from an apple root on which a third instar *Eriosoma lanigerum* had been feeding. (A) Xylem vessel. (B) Parenchyma tissue of the stele. (C) Phloem elements. (D) Endodermis. (E) Parenchyma tissue of the cortex. (F) Epidermis. (G) Cuticle. (H) Trichome. (I) Area of damage caused by *E. lanigerum* feeding. (J) Entry point of stylet.

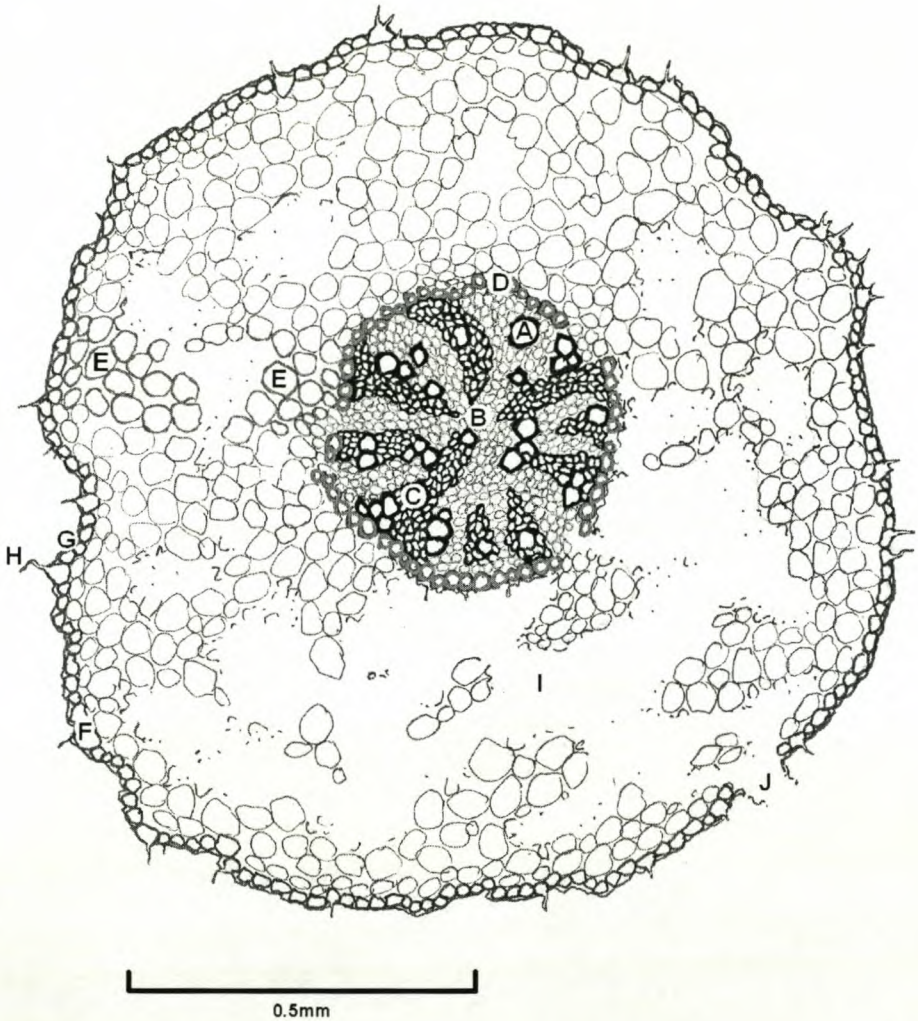

Fig. 2.2.5 Section cut from an apple root on which a fourth instar *Eriosoma lanigerum* had been feeding. (A) Xylem vessel. (B) Parenchyma tissue of the stele. (C) Phloem elements. (D) Endodermis. (E) Parenchyma tissue of the cortex. (F) Epidermis. (G) Cuticle. (H) Trichome. (I) Area of damage caused by *E. lanigerum* feeding. (J) Entry point of stylet.

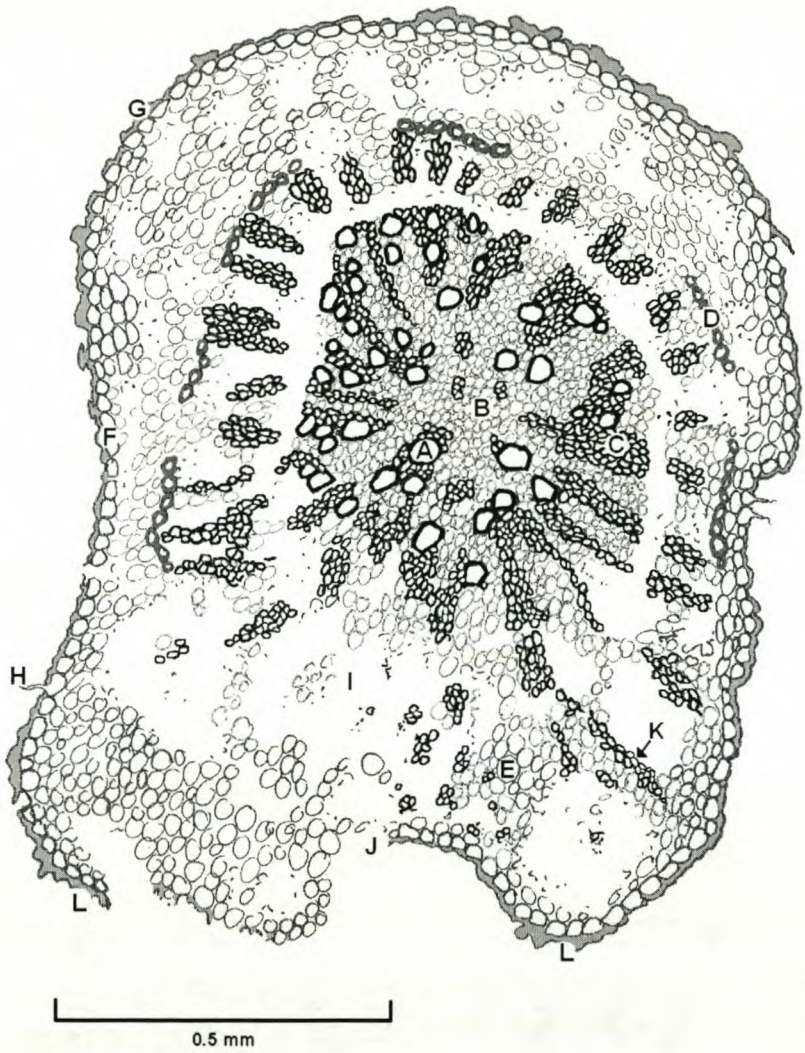

Fig. 2.2.6 Section cut from an apple root on which an adult *Eriosoma lanigerum* had been feeding with the swollen area of the root in the lower half of the figure. (A) Xylem vessel. (B) Parenchyma tissue of the stele. (C) Phloem elements. (D) Endodermis. (E) Parenchyma tissue of the cortex. (F) Epidermis. (G) Cuticle. (H) Trichome. (I) Area of damage caused by *E. lanigerum* feeding. (J) Entry point of stylet. (K) Secondary xylem and phloem. (L) Corky cambium.

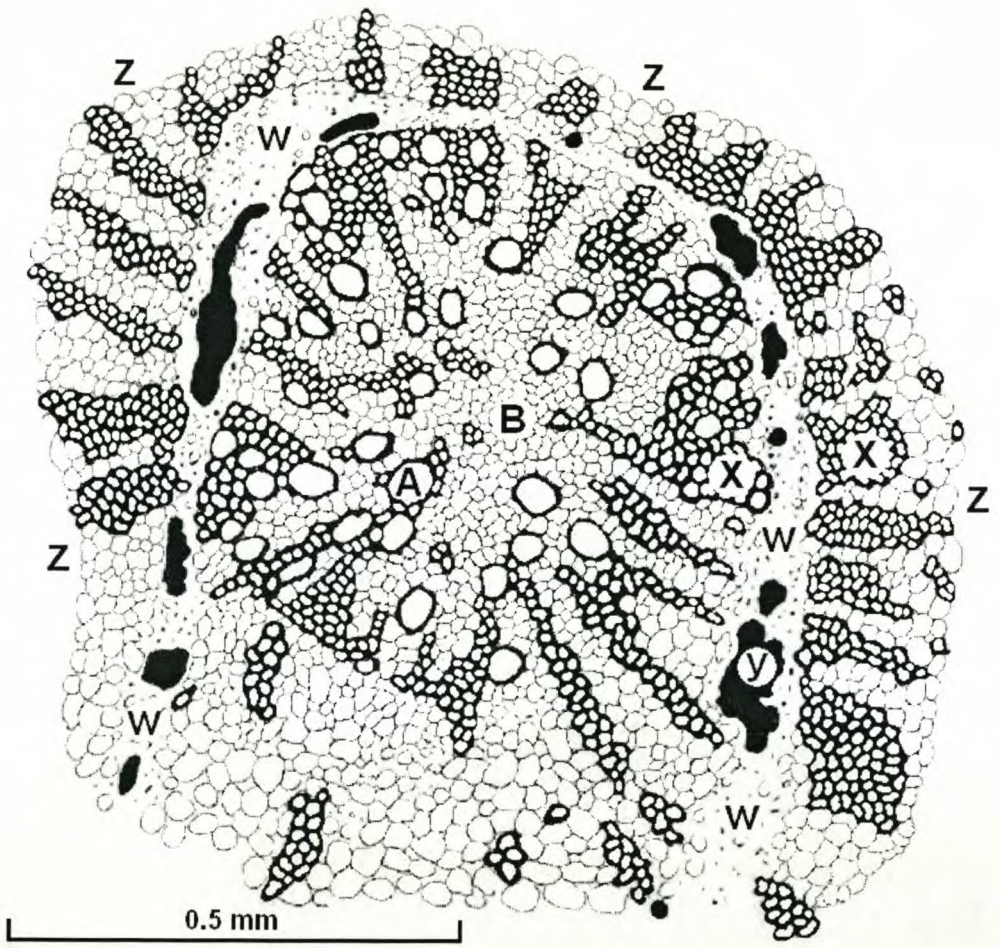

Fig. 2.2.7. Cross-section of apple root core showing damage caused by woolly apple aphid. (A) Xylem vessel. (B) Parenchyma tissue of the stele. (W) Area cleared of tissue by aphid stylet. (X) Vascular ray severed by aphid stylet. (Y) Stylet tissue. (Z) Section of cortex parenchyma tissue not shown

2.3 DISCUSSION

Woolly apple aphid feeding caused blockage of xylem and phloem vessels and caused abnormal xylem growth. In addition, gall tissue contains higher levels of nitrogen than normal root tissue (Brown *et al.* 1991). Roots galled and mechanically damaged through woolly apple aphid feeding, exhibits a five-fold resistance to water flow (Brown *et al.*, 1991). Root tissues used in this study were much younger and therefore contained underdeveloped xylem and phloem vessels (A and C, Fig. 2.2.1). The xylem and phloem tissues did, however, expand into secondary growth and began forming vascular rays at the stage when 5th instar woolly apple aphids were feeding (A and X, Fig. 2.2.7). Woolly apple aphid damage was also assessed after a much shorter period of time than in the experiments of Brown *et al.* (1991). This caused the observed damage to differ from that described by Brown *et al.* (1991) in that early damage was mostly mechanical and restricted to the parenchyma of the cortex (E, Figs. 2.2.1 to 2.2.6).

Older roots frequently lost their epidermis and cortex and developed a corky periderm (Keeton *et al.*, 1993). This was also the case in this experiment where the cuticle (G, Fig 2.2.6) was strengthened by corky cambium tissue (L, Fig. 2.2.6). Staniland (1924) found that penetration by the stylets of *E. lanigerum* was mainly intracellular, but that neither an extracellular nor an intracellular path was possible through a mass of sclerenchyma. The path of damage caused by the aphid stylets in this experiment did not penetrate the vascular bundle until 5th instar (adult) *E. lanigerum* feeding took place. This appeared to be due to the inability of the aphids to pierce the ring of sclerenchyma strengthened endodermis tissue surrounding the vascular bundle (D, Figs. 2.2.1 to 2.2.6).

Brown *et al.* (1991) found that roots with advanced galling consisted mostly of abnormal xylem tissue containing large parenchyma cells, which were not conducive

to water flow. The areas of secondary xylem were found to contain parenchyma cells (K, Fig. 2.2.6) formed in response to structural damage caused by the aphid stylet. These parenchyma cells disrupted water flow. This is a disadvantage for both the plant and the woolly apple aphid.

E. lanigerum disrupted apple root function through feeding damage during the first four instars (Figs. 2.2.2 to 2.2.5), whereafter the root tissues began to visually respond with the formation of secondary tissues (Fig. 2.2.6) which further hampered the flow of nutrients.

It seems that the root tissues of the apple tree respond to aphid feeding by forming dense tissues for protection against further damage and loss of nutrients. This would also explain the absence of *E. lanigerum* on large mature galls (visual observation in orchard).

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CHAPTER 3

INTERACTION BETWEEN THE WOOLLY APPLE APHID AND *Xiphinema* AND *Pratylenchus* NEMATODES IN THE ELGIN AREA

Xiphinema and *Pratylenchus* nematodes are known to attack the roots of apple trees just as *Eriosoma lanigerum* does, causing galling, root dieback and stunting (Wood, 1992; Lan, 1993; Pinochet *et al*., 1994). *Xiphinema* species are also known to be vectors of nepoviruses (Halbrendt, 1993) such as tomato ringspot virus (TmRSV), which cause apple union necrosis (Rosenberger *et al*. 1983). *Pratylenchus penetrans* and *Pythium* spp. can cause apple replant disease (Utkhede *et al*., 1992; Edwards *et al*., 1994). In addition *Pratylenchus vulnus* was thought to be responsible for apple tree decline and decreased yield of apple trees in Israel (Nimrod *et al*., 1999).

In the Grabouw region of the Western Cape Province of South Africa, *Xiphinema* and *Pratylenchus* nematodes and *E. lanigerum* are known to coexist within the rizosphere of trees in apple orchards. The aim of the present study was to determine if these subterranean pests were interacting and since the nature of their interaction was unknown, whether factors such as weather, root growth (measured against nitrogen content) and soil texture influenced their population dynamics.

3.1 MATERIAL AND METHODS

3.1.1 Experimental sites

Samples were collected at three different sites on two different farms in the Elgin area of the Western Cape Province in South Africa. A 4 ha orchard was used at Oak Valley Farm (34°9'S, 19°3'15"E), which was divided into two sampling units of 2 ha each. This orchard was planted in 1978 and consisted of Granny Smith trees and Golden Delicious pollinators, both on seedling rootstock. Only Granny Smith trees were used in the sampling. Two orchards on a second farm, Molteno Trust (34°10'22"S, 19°2'36"E), were also sampled. The orchards were planted in 1970 on M793 rootstock. One of these had alternating double rows of Granny Smith and

Golden Delicious apple trees. Only Granny Smith trees were sampled. The second orchard had only Starking trees. The third and last site consisted of two 2 ha sampling units in an orchard planted in sandy soil (34°7'8"S, 19°3'29"E), also belonging to the Molteno Trust. This will be referred to as Sandy because of the texture of the orchard soil. The orchard was planted in 1994 with alternating triple rows of Royal Gala and Braeburn, both on M793 rootstock. Only the Royal Gala trees were sampled.

3.1.2 Sampling

In each 2 ha sampling unit, 13 evenly spaced blocks of 6 adjacent trees were marked. Each block was sampled at intervals of four weeks but only one of the six trees was sampled on each occasion. Therefore, a sample was taken once in 24 weeks from each tree. This was done so that the roots could recover from sampling.

Sampling for *E. lanigerum* took place from 17 February 1999 to 20 November 2000 at Molteno Trust, 3 March 1999 to 6 November 2000 at Oak Valley and 15 March 1999 to 6 November 2000 at Sandy. Sampling for nematodes was conducted from 7 June 1999 to 6 November 2000 at Molteno Trust, 19 July 1999 to 6 November 2000 at Oak Valley and 5 July 1999 to 20 November 2000 at Sandy.

3.1.3 Auger

The auger used to take *E. lanigerum* samples was constructed from metal piping with an internal diameter of 5.0 cm. It had an external collar 15 cm from the tip, which restricted penetration beyond this depth. There were two metal handlebars welded to the top. These were used to rotate the auger while exerting downward pressure causing it to penetrate the soil.

3.1.4 Sampling methods

Woolly apple aphid samples were collected using the soil auger (3.1.3 Auger) at a distance of 30 cm from the tree trunk. One such sample was taken from one tree at each of the 13 sampling sites per 2 ha sampling unit.

Nematode samples were collected using two different methods, one for each nematode genus. The presence of typical lesions on the roots at Oak Valley suggested that there was an infestation of *Pratylenchus* nematodes at this site. *Pratylenchus* nematodes are internal parasites and roots were therefore sampled. Samples from this site contained at least 5 g of fine roots with as little soil as possible. Roots were dug out using a small garden shovel, taking care to select only fine roots (diameter less than 5 mm). These were placed in plastic bags. *Pratylenchus* was not sampled at the other sites.

Xiphinema are free-living external parasites and were sampled by collecting soil with or without roots. They were collected with a hand spade from four different points within a 0.5 m radius around the tree trunk to a depth of up to 20 cm. Nematode samples from Molteno Trust and Sandy contained at least 500 ml of soil, of which 250 ml was used.

All samples were transported in labeled plastic bags inside insulated cooler bags for protection against heat and desiccation. Nine nematode samples were collected per visit from the first nine sample blocks in each 2 ha sampling unit.

3.1.5 Treatment of *Eriosoma lanigerum* samples

Soil samples were stored in a refrigerator at 5 °C. Woolly apple aphid samples were rinsed through two sets of sieves. An ordinary large kitchen sieve separated large objects from the rest of the sample. Aphids and smaller particles were collected in the second sieve with a mesh size of 200 µm, small enough to prevent crawlers from passing through (Damavandian & Pringle, 2002). Clay particles and small fragments were rinsed through the sieve. The sieve was placed in a shallow container (1.5 cm deep) in which there was a sugar solution (600 g sucrose/l water). The aphids floated to the surface of the sugar solution, facilitating counting.

All developmental stages counted were classified as dry, dead or alive. Those classified as dry were disregarded from the data analysis, as they were presumed to have died prior to sampling. *E. lanigerum* classified as dead were still turgid. They

were assumed to have died during sampling, transport, storage or washing. Soil samples taken for determining aphid populations were classified into five categories: no roots, roots with no visible damage, roots with less than 25 % damage, roots with between 25 % and 50 % damage and roots with more than 50 % damage. Spearman's rank correlation was used to determine whether or not there was a relationship between the five root categories and number of woolly apple aphids in the soil.

3.1.6 Identification of nematodes

The *Xiphinema* nematodes were identified by Prof. A. J. Meyer of the Department Entomology and Nematology at the University of Stellenbosch, South Africa. The *Pratylenchus* nematodes were sent to the Agricultural Research Council (ARC) in Pretoria, South Africa for identification.

3.1.7 Treatment of Nematode samples

A modified Baerman technique was used to extract nematodes (Southey 1986). Root samples taken for studying *Pratylenchus* were first washed in water and scrubbed with a fine brush to remove as much soil as possible. Each sample was then macerated using a commercial blender for 5 to 10 seconds. Five grams of the macerated roots were spread onto a 45 µm sieve and left for 24 hours on a 25 cm diameter plate containing 100 ml of water. The sieves were kept clear of the plate floor allowing the nematodes to filter through into the plate. After 24 hours each sieve was carefully removed, rinsing the contents into the plate. The fluid in the plate was drained into a 100 ml beaker and left to settle for at least an hour before the top 80 ml of the liquid was decanted. The remaining 20 ml of fluid and sediment in the beaker was poured into a petri dish before the *Pratylenchus* nematodes were counted using a stereo microscope with base lighting.

Soil samples taken for studying *Xiphinema* were thoroughly mixed before 250 ml of soil was extracted and rinsed through an ordinary large kitchen sieve to remove large particles. The remainder was rinsed three times through a series of four bowls, each time collecting nematodes in a 45 µm sieve. The sieves containing the nematodes were left for 24 hours in 25 cm diameter plates containing 100 ml of water to cover each sieve with a film of water. The rest of the process was identical to that described for the *Pratylenchus* samples.

Symptoms ascribed to *Xiphinema* damage included severe blackening of roots, stunted secondary roots with severe stubby, branching rootlets and numerous dead and dry rootlets as well as areas where the external diameter was reduced. Soil samples were classified into four categories, no roots, roots with no visible damage, roots with less than 50 % damage and roots with more than 50 % damage. Spearman's rank correlation was used to determine whether or not there was a relationship between the five root categories and number of *Xiphinema* in the soil.

3.1.8 Root samples for nitrogen determination

Root samples collected from Oak Valley and Molteno Trust for nitrogen determination consisted of three samples per 2 ha sampling unit. There was an undamaged root sample, a galled root sample and a sample consisting of undamaged roots between galls. They were collected once every month on the same dates as samples were taken for *E. lanigerum*. Every third tree used for *E. lanigerum* sampling was used for sampling roots for nitrogen determination during each visit, giving four root samples on each sampling date (13 six tree blocks were sampled for *E. lanigerum*, 3.1.2 Sampling). For example, if blocks 1, 4, 7 and 10 were sampled for nitrogen in roots on a certain date, then blocks 2, 5, 8 and 11 would be sampled on the next visit. Using this system root samples were not taken more than once from the same tree during the study.

The root samples taken at each visit from these four tree blocks were then pooled into three composite samples, one for each root kind. Each pooled sample of undamaged roots, galled roots and undamaged roots between galls was regarded as a treatment while every date of sampling was regarded as a replicate. Preliminary sampling indicated that the woolly apple aphid population levels at Sandy were low. Therefore, root samples for nitrogen determination were not taken at this site, as there were insufficient galled roots.

Samples collected for nitrogen determination were sent to BEMLAB, Somerset West for nitrogen analysis (% nitrogen by dry weight). A one way analysis of variance was used to examine the resulting data. Means were compared using Bernoulli's inequality to compensate for multiple comparisons at a significance level of $P = 0.05$.

3.1.9 Soil analysis

Twenty-six (one from each of the 13 sampling sites per 2 ha sampling unit) sub-samples of soil were collected from each orchard. One was taken from each sampling tree 30 cm from the tree trunk and at a depth of no more than 25 cm. The

sub-samples for each orchard were thoroughly mixed together to form a pooled sample, which was sent for particle analysis (including the percentage stone by volume) by BEMLAB, Somerset West. One pooled sample was taken for analysis from each of Oak Valley, Molteno and Sandy.

Stone was classified as particles with a diameter of > 2 mm for the purpose of this analysis. Particles with a diameter > 2 mm can also be classified as rough particles, which could be subdivided into gravel (diameter between 2 mm and 75 mm) and stone (diameter between 75 mm and 250 mm). The greater part of the stone fraction had a diameter in the range of 10 mm to 100 mm. Course sand has by definition a diameter between 0.5 mm and 2 mm, medium sand between 0.25 mm and 0.5 mm, fine sand between 0.05 mm and 0.25 mm, loam between 0.002 mm and 0.05 mm and clay particles have a diameter less than 0.002 mm (MacVicar, 1991).

3.1.10 Weather data

Temperature and precipitation data from a weather station in Elgin (34°10'49''S, 19°02'39''E) were used. The environmental data used were the maximum and minimum temperatures measured in °C and the monthly precipitation measured in mm/day over the course of almost two years from February 1999 to November 2000.

3.2 RESULTS AND DISCUSSION

The *E. lanigerum* population levels from Molteno Trust (Fig. 3.2.1) peaked in mid March 1999 before declining during the colder wetter months (May to August 1999, Table 3.2.1). Numbers were low until mid February 2000 when they increased and remained high until mid May 2000. *E. lanigerum* numbers declined again during the winter months of June and July 2000 before increasing in early August 2000. They remained constant until mid November 2000.

The *Xiphinema* nematodes were identified as *X. americanum*. Their numbers at Molteno Trust remained low for most of the study period (Fig. 3.2.1). However, from July to October 2000 there was a sudden increase in numbers, followed by a rapid decline in early November 2000.

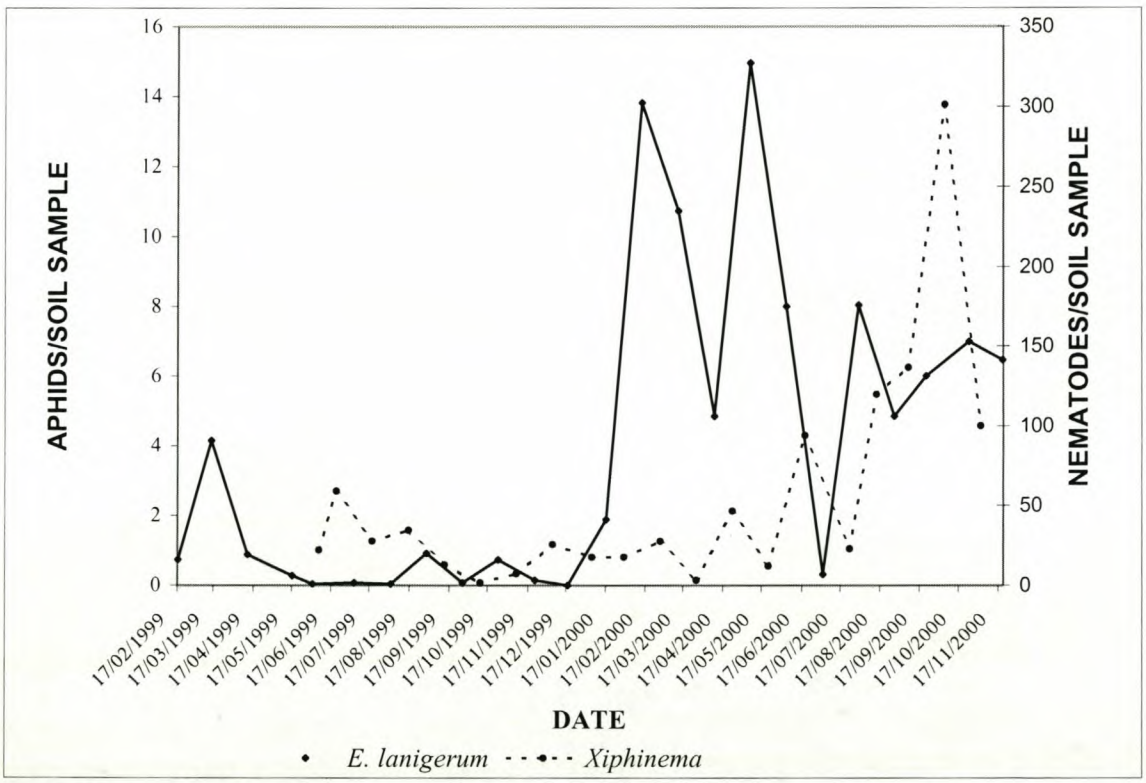

Fig. 3.2.1: *Eriosoma* and *Xiphinema* population levels for Molteno from February 1999 to November 2000.

Table 3.2.1: Monthly average maximum and minimum temperature and precipitation for Elgin from February 1999 to November 2000.

Date	Average maximum °C	Average minimum °C	Monthly precipitation (mm)
February-99	26.5	16.1	0
March-99	26.5	14.7	10.2
April-99	22.8	12.2	84.2
May-99	19.3	9.6	97.4
June-99	19.7	6.7	130.2
July-99	17.3	5.8	176.4

August-99	18.3	7.7	196.4
September-99	16.8	7.7	146.8
October-99	20.7	10.3	19
November-99	23.4	12.1	45.2
December-99	28.7	16.6	30.8
January-00	27.1	14.9	66.1
February-00	26.6	15.4	5.3
March-00	24.2	14.8	53.7
April-00	23.2	10.8	22.2
May-00	20.3	8.1	38.86
June-00	19.4	7.1	111.6
July-00	17.5	5.6	205.6
August-00	18.1	8.2	118.6
September-00	17.5	7.4	190.8
October-00	21.4	9.4	30.8
November-00	23.8	12.1	26.2

The *E. lanigerum* population on Oak Valley (Fig. 3.2.2) followed a similar pattern to that of the woolly apple aphid population on Molteno Trust with low numbers during the winter and higher numbers from spring to autumn.

The *Pratylenchus* nematodes were classified as *Pratylenchus penetrans*. There was no pattern in the *P. penetrans* population levels, which fluctuated erratically throughout the study period (Fig. 3.2.2).

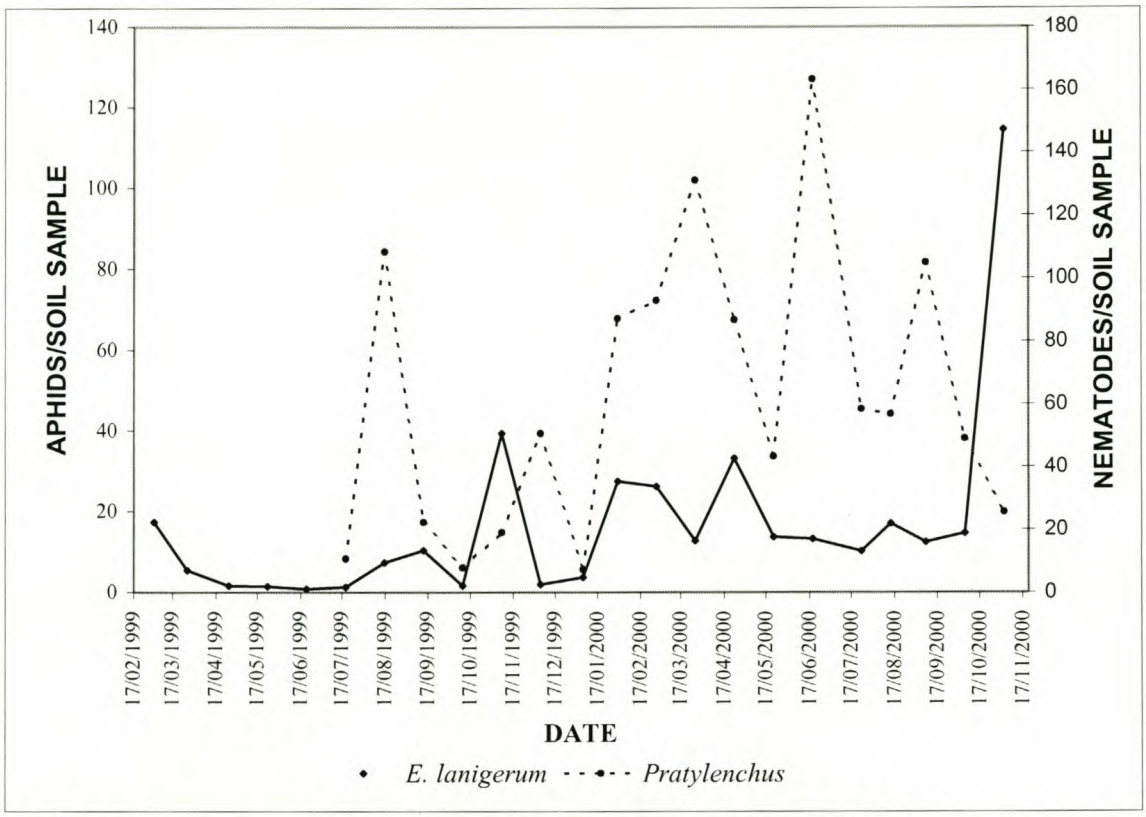

Fig. 3.2.2: *Eriosoma* and *Pratylenchus* population levels from Oak Valley from February 1999 to November 2000.

The *E. lanigerum* population level from Sandy was low throughout the study period (Fig. 3.2.3). It reached a peak during the autumn of both years. The *Xiphinema* population on Sandy (Fig. 3.2.3) remained low for most of the study period, but reached peak numbers during late October 2000, the same time that *Xiphinema* numbers from Molteno Trust also increased (Fig. 3.2.1).

There was no apparent relationship between *E. lanigerum* and *Xiphinema*. The occurrence of the former appeared to be seasonal at both Molteno and Sandy, while there was no apparent pattern in the case of the latter, as numbers remained low for most of the study period, increasing suddenly towards the end at both sites. There also appeared to be a seasonal cycle in *E. lanigerum* numbers at Oak Valley (Fig. 3.2.2),

while *P. penetrans* numbers fluctuated erratically. Therefore, there did not seem to be a relationship between these two root feeding organisms at Oak Valley either.

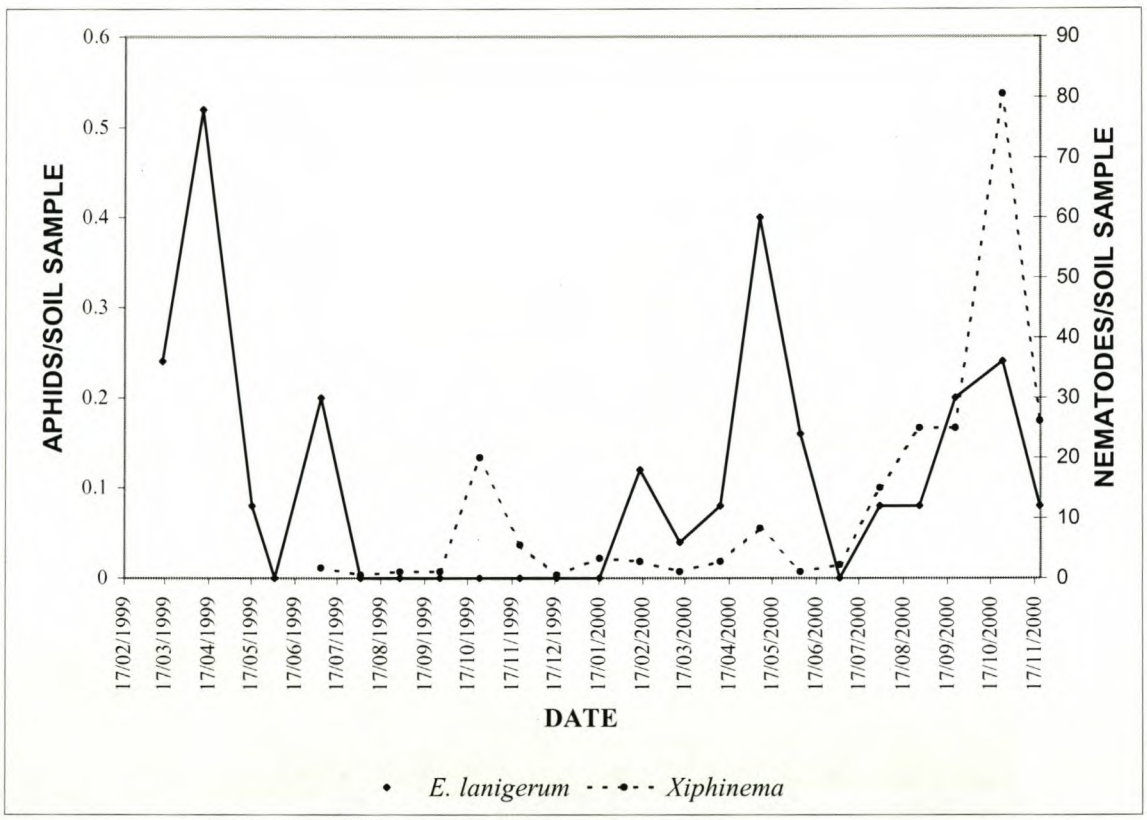

Fig. 3.2.3: *Eriosoma* and *Xiphinema* population levels from Sandy from March 1999 to November 2000.

Root nitrogen levels on Molteno (Fig. 3.2.4) and Oak Valley (Fig. 3.2.5) seemed to correspond with the normal root growth cycle. High root nitrogen levels normally occurred during spring and autumn when roots are actively growing (Tusten *et al.* 1997, Iivonen *et al.* 2001).

The nitrogen levels from undamaged roots from Molteno (Fig. 3.2.4) fluctuated constantly although nitrogen levels during spring and autumn each year were usually higher than at other times. This was indicative of root growth flushes (Loubser, 1993).

There were significant differences ($P < 0.05$) between the nitrogen levels of the galled roots (mean 0.809 %, Fig. 3.2.4) and those of the undamaged roots (mean 1.127 %,) as well as between those of the galled roots and roots taken from between galled areas (mean 1.135 %). There was no significant difference between nitrogen levels from undamaged roots and those of roots taken from between galled areas ($P > 0.05$). The nitrogen levels of roots taken from between galled areas fluctuated sharply, increasing during winter and decreasing during spring and autumn (Fig. 3.2.4). The similarity of the nitrogen levels from roots taken from between galls to those of undamaged roots could have resulted from accumulating nutrients trapped between the galled areas due to disjointed xylem and phloem development, preventing abnormally low nitrogen status. The consistently lower root nitrogen levels from galls were probably due to *E. lanigerum* feeding.

Table 3.2.2: Analysis of variance table comparing % nitrogen per dry weight of apple roots taken at Molteno from undamaged roots, galled roots and undamaged roots between galls.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Areas	1.664065	2	0.832033	15.72126	< 0.001
Within Areas	3.651758	69	0.052924		
Total	5.315823	71			

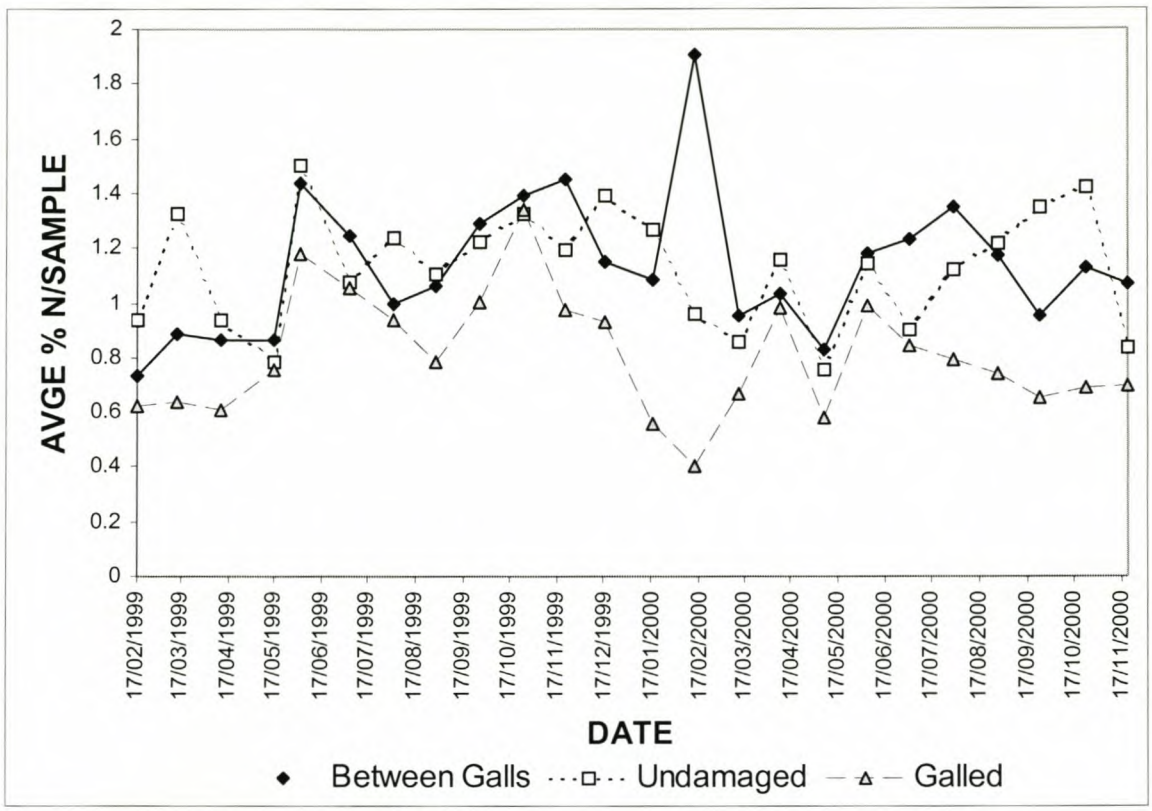

Fig. 3.2.4: Average % nitrogen found in three types of orchard roots from Molteno from February 1999 to November 2000.

Root nitrogen levels from undamaged roots taken from Oak Valley (Fig. 3.2.5) showed a similar pattern to those of undamaged roots from Molteno (Fig. 3.2.4). Root nitrogen levels from roots taken from between galled areas increased in spring, early summer and autumn each year. Root nitrogen levels from galls were significantly lower ($P < 0.05$, mean 0.655 %, Fig. 3.2.5) than those of the undamaged roots (mean 0.785 %). There was no significant difference in nitrogen levels between galled roots and roots between galled areas (mean 0.748 %) ($P > 0.05$). There were also no significant differences between the root nitrogen levels from roots taken from between galled areas and from undamaged roots (Table 3.2.3).

Table 3.2.3: Analysis of variance table comparing % nitrogen per dry weight of apple roots taken at Oak Valley from undamaged roots, galled roots and undamaged roots between galls.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	0.207972	2	0.103986	5.846203	0.005
Within Groups	1.173936	66	0.017787		
Total	1.381908	68			

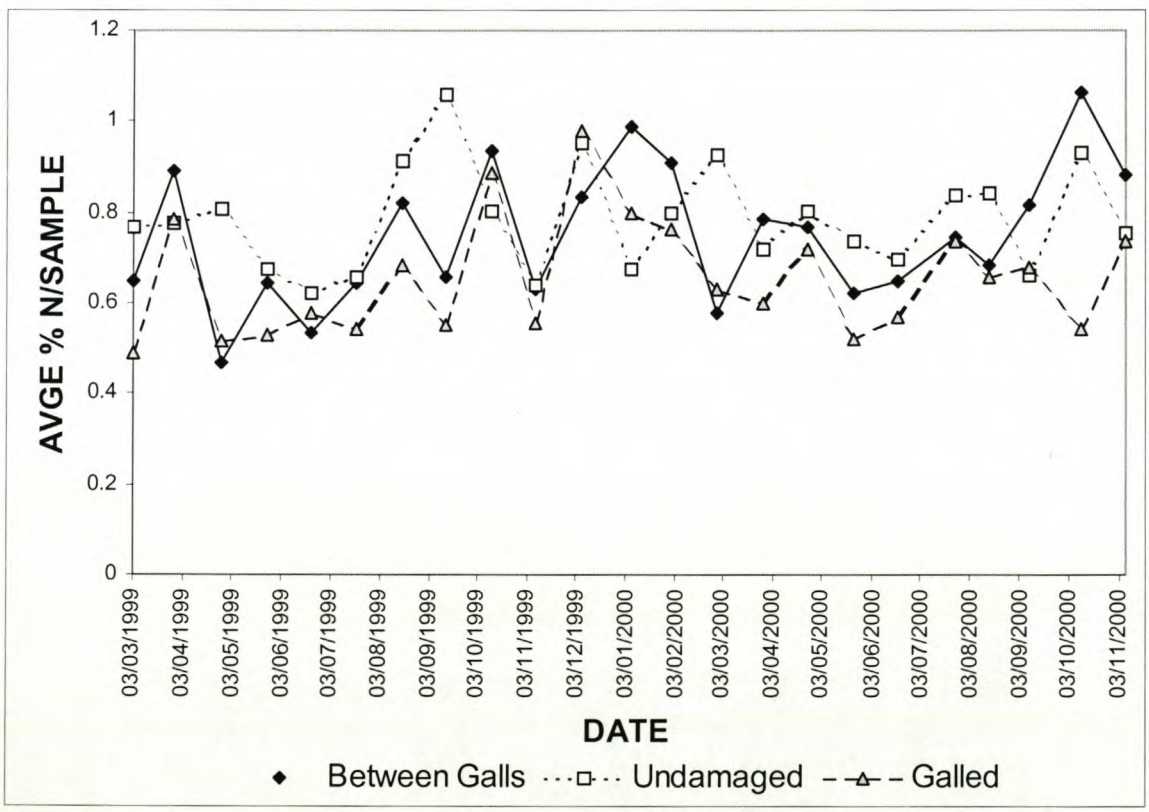

Fig. 3.2.5: Average % nitrogen found in three types of orchard roots from Oak Valley from February 1999 to November 2000.

The soil found at Sandy (Fig 3.2.6) contained considerably more course and medium sand and less stone than soil from the other two sites, and was classified as sand (Sa). Orchard soil from Oak Valley contained mainly stone and fine sand. It was classified as sand-clay-loam (SaClLm). Soil from Molteno contained mainly stone.

The remainder consisted mostly of fine sand and clay. This soil was classified as loam (Lm). It is uncertain whether or not the soil types influenced the population levels of the organisms. However, there were fewer *E. lanigerum* in the sandy soils at Sandy than in the soils containing more fine sand and clay at Oak Valley and Molteno (Fig. 3.2.7). In addition, *Xiphinema* numbers were higher in the soil containing more fine sand and clay (Molteno) than in the sandy soil (Sandy).

It seems therefore that soils containing greater fractions of fine sand and clay sustained higher populations of *E. lanigerum* and *Xiphinema*. Soils rich in clay have a greater amount of fine particles, enabling the soil to trap more free water in capillary cavities between particles. Dry soil and heat favours *E. lanigerum* as population numbers are always higher in the warmer, drier months (Figs 3.2.1, .3.2.2 and 3.2.3). Soils rich in clay expand and contract more as they moisten and dry than other soils leading to a greater development of vertical and horizontal cracks in the soil. *E. lanigerum* nymphs and adults would be able to move more freely through such cracks than in a soil with more sand which does not crack.

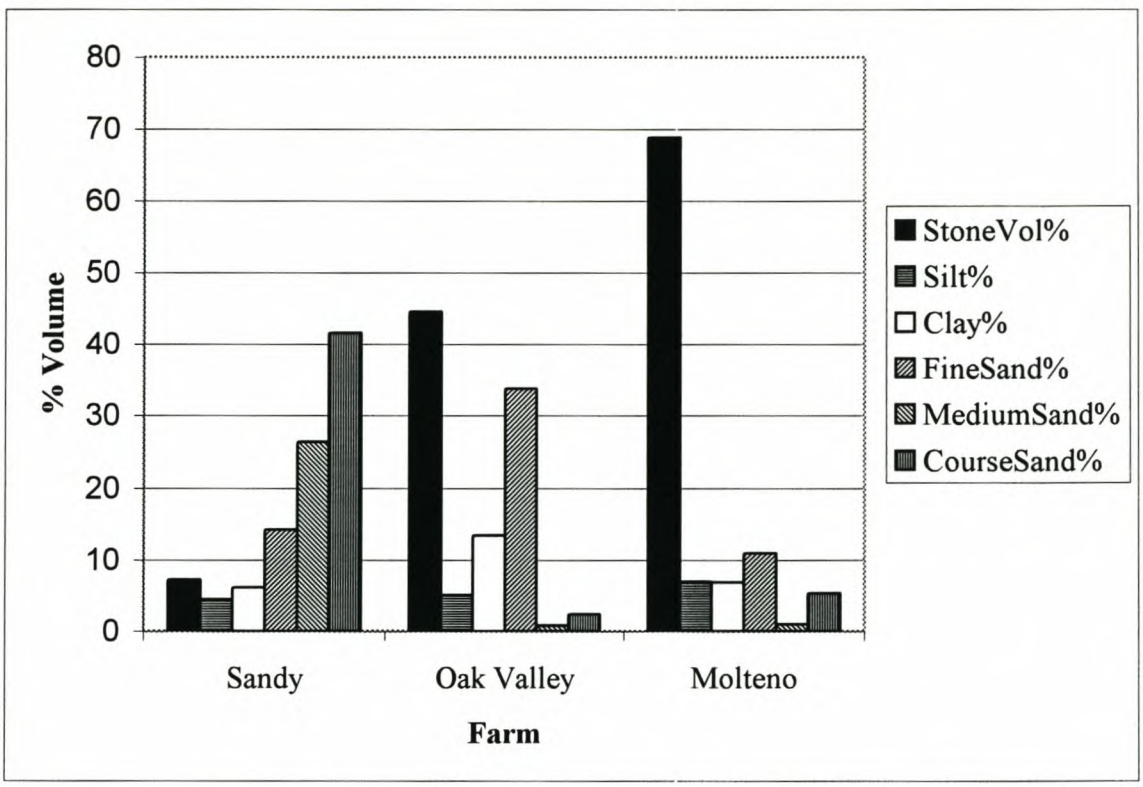

Fig. 3.2.6: Soil fractions by percent volume for Sandy, Oak Valley and Molteno

There was a good correlation between the number of woolly apple aphids found in the soil samples (Fig. 3.2.7) and the damage index allocated to the samples ($r_s = 0.83$; d.f. = 13; $P < 0.001$). This supports the findings of Damavandian & Pringle (2002). There was also a good correlation between the number of *Xiphinema* found in the soil samples (Fig. 3.2.8) and the index allocated to the samples according to suspected damage symptoms ascribed to *Xiphinema* ($r_s = 0.84$; d.f. = 5; $P = 0.017$). Although not conclusive, these results suggest that the damage symptoms ascribed to *Xiphinema* may well be true.

There was no apparent relationship between *E. lanigerum* and *Xiphinema* numbers at either Molteno Trust or Sandy nor between *E. lanigerum* and *P. penetrans* numbers at Oak Valley.

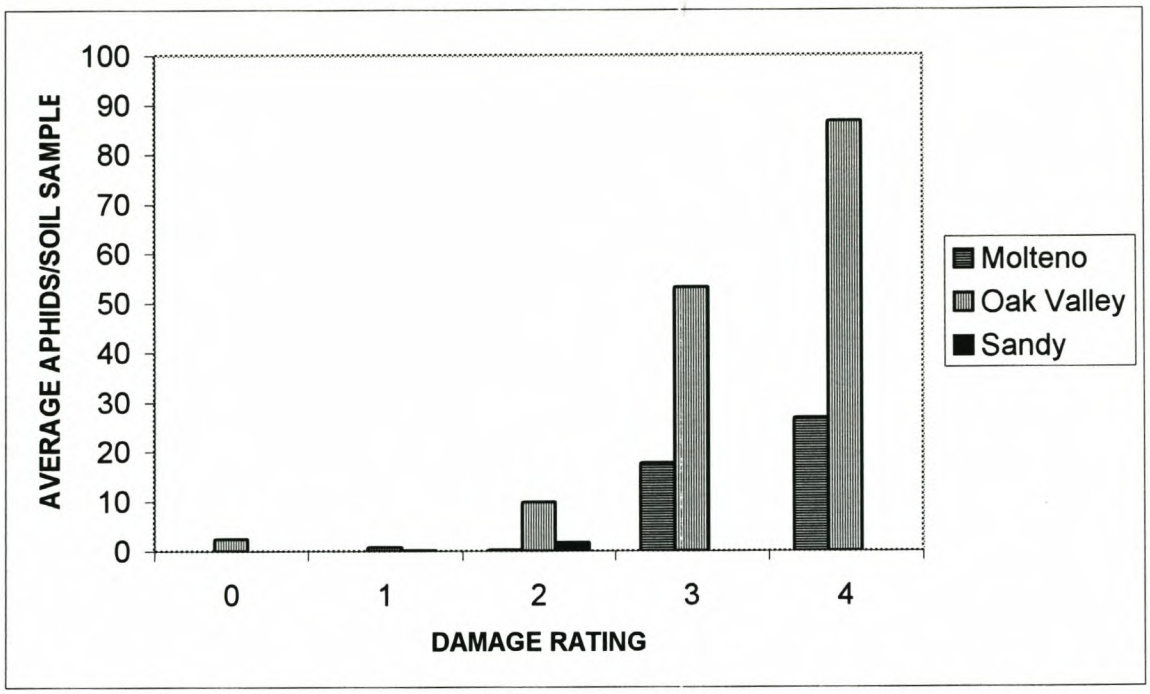

Fig. 3.2.7: Average number of *E. lanigerum* per soil sample from Molteno, Oak Valley and Sandy for different damage ratings

Galled roots had significantly lower nitrogen levels than undamaged roots at both Molteno and Oak Valley, probably due to *E. lanigerum* feeding. Galled roots also had significantly lower nitrogen levels than roots taken from between galled areas at Molteno, but not at Oak Valley

Soils with high fractions of fine sand and clay sustained greater populations of *E. lanigerum* and *Xiphinema* than sandy soils. The damage indexes awarded to E. lanigerum and Xiphinema samples seemed to be indicative of the numbers present.

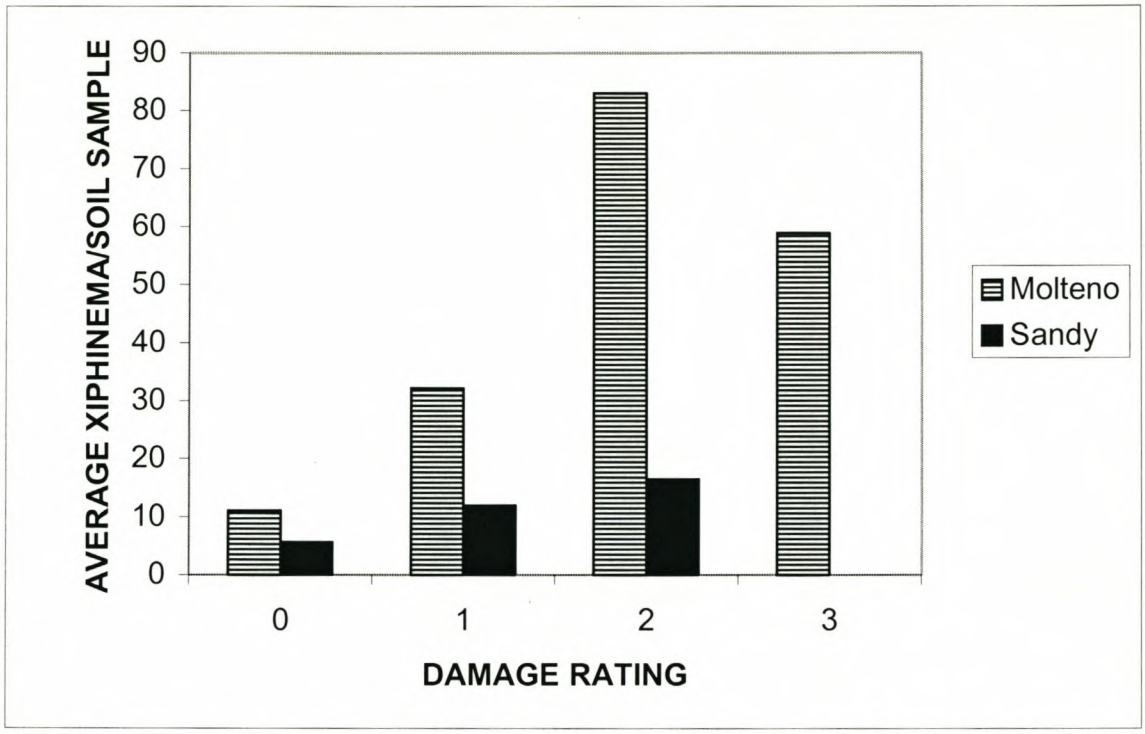

Fig. 3.2.7: Average number of *Xiphinema* per soil sample from Molteno and Sandy for different damage ratings

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CHAPTER 4

THE EFFECT OF BIOSTART 2000® AND FURFURAL® ON WOOLLY APPLE APHIDS ON POTTED APPLE TREES

Subterranean woolly apple aphid, *Eriosoma lanigerum* (Hausmann), attacks the roots of apple trees, causing galls which restrict water and nutrient movement (Brown *et al.* 1991). Several methods have been used to control *E. lanigerum* since its discovery in South African orchards in 1895 (Anneke & Moran, 1982). Fuller (1904) recommended the application of near boiling water and tobacco dust around the tree trunk. Migrating crawlers were trapped in grease bands applied by Greenslade (1936). More recently, it has been controlled using pesticides such as vamidothion in cover sprays (Pringle *et al.* 1994) and imidacloprid as a soil treatment (Pringle 1998). However, some strains of *E. lanigerum* have developed tolerance to vamidothion (Pringle *et al.* 1994). Experiments with straw mulches have shown promise for woolly apple aphid control, but there are limited supplies of straw (Damavandian 2000).

Staniland (1924), Knight *et al.* (1962), Rock & Zeiger (1974) and Taylor (1981) have identified apple rootstocks resistant to *E. lanigerum*. However, a strain of woolly apple aphid has been found which has overcome the resistance factor in Northern Spy and related rootstocks in South Africa (Giliomee *et al.* 1968). Therefore, very few options for the control of woolly apple aphids are available locally.

The aim of the present study was to conduct preliminary tests for the use of a mixture of bacteria, Biostart 2000®, and a by-product from the local sugar industry, Furfural®($C_5H_4O_2$), for the control of subterranean woolly apple aphids.

4.1 MATERIAL AND METHODS

4.1.1 Trees and experimental conditions

The apple trees (*Malus domestica*) used were one year old Granny Smith trees on M5 rootstock. They were kept in cold storage at 4 °C for three months to satisfy their cold requirements, after which they were planted in 10ℓ pots in commercial sterile potting soil. The trials were conducted under laboratory conditions at room temperature and 14 hours of light each day. The soil was kept moist by frequent watering. The trees were allowed to sprout and when sufficient hardening of the new growth had occurred, the roots were infected with *E. lanigerum* crawlers.

4.1.2 Collection and treatment of aphids

Woolly apple aphid infected roots were collected from Oak Valley farm (34°9'S, 19°3'15"E) in the Grabouw district of the Western Cape Province, South Africa. Adults were carefully removed from the roots with a small brush and placed on filter paper in petri dishes. The petri dishes were placed in an incubator at 23 °C and 80 % relative humidity (RH) in the dark. After 24 hours crawlers produced by these adults were removed and 50 were placed on the exposed roots of every potted tree. The surface of each pot was covered with a circle of black PVC plastic wrapped around the trunk of the tree to provide dark conditions. The crawlers were allowed to settle and were inspected each day to ensure adequate infection.

4.1.3 Treatment of trees

There were three treatments, Biostart 2000®, Furfaral® and a control treated with water. The treatments were replicated 10 times in a one-way design. Therefore a total of 30 trees were used. Details of the composition and properties of Furfural® and Biostart 2000® are given in the appendix. Biostart 2000® consists of two compounds,

a microbial soil inoculant and a microboost activator, which produce a reactive bacterial solution when mixed. The microbial soil inoculant is a thick syrupy mixture of three different *Bacillus* species, *B. chitinosporus*, *B. laterosporus* and *B. licheniformis* and also contains cobalt, molybdenum and sodium. The microbial strength is between a thousand million and 1 billion colony forming units (CFU) per ml and the product does not function at a pH less than 5.0. The microboost activator is in powdered form and is mixed with the microbial soil inoculant at 1 g: 1 ml. The resulting paste can be thinned with water to enable the Biostart 2000® to wash through the soil to the root zone of the trees.

The recommended application rate for Biostart 2000® is 2 ℓ per ha, applied as a 1 % solution. Therefore, a 1 % solution was made up and this was applied at 0.454 ml/pot, as the surface area of the pots was 0.0227 m^2.

The recommended application rate of Furfural® is 200 ℓ/ha applied as an 8 % solution. Therefore, in this case 5.675 ml of an 8 % solution of Furfural® was applied per pot. These applications were made one week after infestation. In each case the solutions were washed into the soil with water immediately following application. Care was taken to avoid over watering so that the applications were not washed out of the pots. The control trees were treated with water.

The trees were kept under the aforementioned conditions for a month (31 days) and were regularly watered, but never with more than the water holding capacity of the soil, as this would cause the treatments to be washed out of the root zone, making the treatment less effective.

4.1.4 Assessment methods

After one month, each tree was taken from its pot and all the soil was washed from its roots and from the pot through two sieves. The larger sieve was an ordinary large kitchen sieve and was used to remove large objects. The second sieve had a mesh size of 200 μm, just large enough to keep the first instar aphids from washing through the sieve, but sufficiently large for fine clay particles to pass through the sieve. The finer sieve was mounted in a rectangular stainless steel frame, which fitted snugly inside a rectangular stainless steel container. This container was filled with a sugar solution (600 g sucrose/litre water) with a higher density than pure water causing the aphids to float, which facilitated counting. The container and sieve were placed under a microscope and the aphids counted. Aphids from each tree (repetition) were classified as dry (long dead), dead (assumed to have been killed in the process of washing the soil) or alive. Aphids classified as dry were not included in the analysis as they were assumed to have died prior to being extracted from the soil.

4.1.5 Data analysis

A one-way analysis of variance was used. The variances using the raw data were heterogeneous. Therefore, the original data were transformed using log(X+1) to stabilize the variances. Means were compared using Bernoulli's inequality to compensate for multiple comparisons.

4.2 RESULTS

There were large differences between treatments (Fig. 4.2.1, Table 4.2.1). There were more aphids in the soil treated with water (mean 48.8) than in the soil treated with Biostart 2000® (mean 1.9) and Furfural® (mean 7.2) (Fig. 4.2.1). The Furfural® treated potted trees had more aphids than the Biostart 2000® treated trees ($P < 0.05$). However, this difference was substantially smaller than the difference between the water treated control trees and the trees receiving the other two treatments (Fig. 4.2.1).

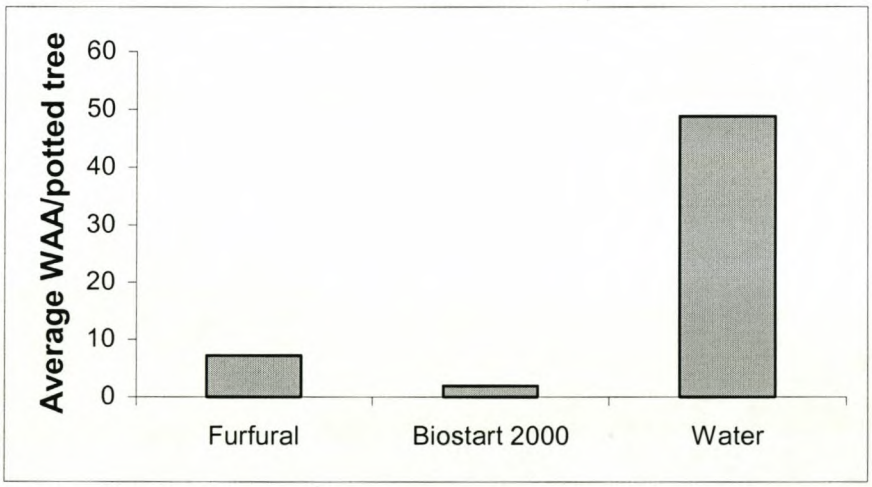

Fig.4.2.1 Average number of woolly apple aphids (WAA) per potted tree treated with Furfural®, Biostart 2000® or water.

Table 4.2.1 Analysis of variance table comparing number of aphids counted in the soil from potted apple trees treated with Biostart 2000®, Furfural® and water. The data were transformed using log(X+1).

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Treatments	10.99903	2	5.499514	27.40034	3.17E-07
Within Treatments	5.419161	27	0.20071		
Total	16.41819	29			

4.3 DISCUSSION

Both materials tested reduced the number of aphids relative to the water treated control. However, the Biostart 2000® was more effective than the Furfural® and this may possibly be due to the fact that Biostart 2000® is a bacterial preparation. The bacteria can multiply and spread through the soil and the aphid population, which is not the case with Furfural®.

Biostart 2000® is much easier to prepare than Furfural® since the soil inoculant and the microboost activator comprising Biostart 2000® readily mix, forming a paste easily thinned with water. However, Furfural® is an oily substance that forms a suspension in water with difficulty. Furfural® is sold in 245 kg to metric ton volumes at R 9955.00/metric ton (excluding value added tax or VAT at 14 %). At a recommended application rate of 200 ℓ/ha it would cost R 1991.00/ha for a single application. Biostart 2000® is sold in 5 ℓ bottles at R 850,00 per bottle (excluding VAT) and at a recommended application rate of 2 ℓ/ha would cost R 340.00/ha. All prices were obtained during November 2002. Furfural® is a byproduct of the sugarcane industry and is therefore surprisingly less cost effective to obtain in large volumes than Biostart 2000®, especially since delivery costs were not included.

Biostart 2000® seemed to be the better treatment for the control of E. lanigerum as it was more effective, easier to apply and more cost effective than Furfural®.

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APPENDIX

Biostart 2000® is a bacterial product intended for use against a variety of subterranean pest organisms including nematodes (Daneel *et al.* 1998). It consists of an inoculum mixture of *Bacillus licheniformis*, *B. laterosporus* and *B. chitinosporus*. There is more information available on *B. licheniformis* than on *B. laterosporus* and *B. chitinosporus*.

B. licheniformis has nematicidal effects (Marquez *et al.* 1997). This was demonstrated through the successful control of plant parasitic nematodes attacking banana using Biostart 2000® (Daneel *et al.* 1998). *B. licheniformis* is a facultatively anaerobic bacterium (Inglis *et al.* 1998) that grows and reproduces under a wide range of temperatures, and in the presence of high concentrations of salts (Lin *et al.* 1994). It has bactericidal qualities and produces amoebicin (Gálvez *et al.* 1994; Lebbadi *et al.* 1994a; Lebbadi *et al.* 1994b; Lebbadi *et al.* 1995), cell wall lytic enzymes (Kuroda *et al.* 1992), proteases (Otte *et al.* 1997) and chitin binding peptides (Oita *et al.* 1996).

Queiroz & Boyd (1998) demonstrated the beneficial effects of Biostart 2000® and *B. licheniformis* on the growth and survival rate of catfish, *Ictalurus punctatus*. However, the reason for the enhanced fish growth facilitated by the bacteria remains unexplained. *B. licheniformis* also possesses high keratinase activities, degrading chicken waste high in feather content (Lin *et al.* 1992; Nitisinprasert *et al.* 1999). Kalia *et al.* (1994) also demonstrated that *B. licheniformis* could reduce biowaste to H_2 through fermentation. *B. licheniformis* effectively controlled anthracnose on mangoes when applied post harvest (Korsten *et al.* 1992).

However, toxigenic strains of *B. licheniformis*, harmful for human and animal consumption also exist (Wei *et al.* 1996). Harmful levels have been found in products such as margarine and yogurt (Gonzales *et al.* 1998), raw milk and baby food (Meer *et al.* 1993; Salkinoja-Salonen *et al.* 1999) and snack and lunch foods (Varadaj *et al.*

1992). Strains of *B. licheniformis* have been found to cause food poisoning through enterotoxins (Beattie & Williams 1999) septicemia, peritonitis and opthalmitis (Salkinoja-Salonen *et al.* 1999). *B. licheniformis* has also been identified as an agent responsible for abortion in water buffalo (*Bubalus bubalis*) (Galiero & De-Carlo 1998) and diary cows (Hommez *et al.* 1997) through histopathological lesions (Johnson *et al.* 1994). The infection of immunodepressed mice with *B. licheniformis* caused brain and pulmonic lesions (Agerholm *et al.* 1997), which have sometimes been fatal (Wei *et al.* 1996).

B. laterosporus has antifungal qualities (Rosales *et al.* 1993) as well as an insecticidal action. It exhibits larvicidal activity against mosquitoes through spores and crystalline inclusions (Rivers *et al.* 1991; Orlova *et al.* 1998). It has, however, also been reported to be associated with food borne diseases (Beattie & Williams 1999), and has been found in products such as dried market spices, condiments (Nkama *et al.* 1994), snack foods (Varadaj *et al.* 1992) and raw milk (Meer *et al.* 1993).

B. chitinosporus produces the enzyme chitinase, which biodegrades chitin of nematode eggs and the exoskeleton of adult, larval and pupal insects. *B. chitinosporus* was among bacteria isolated from soil treated with a 1% chitin solution. The changes in the bacterial community eliminated plant-parasitic nematodes in a first planting of cotton (*Gossypium hirsutum* L.) cv. 'Rowden' and severely reduced *Meloidogyne incognita* infestation in a second planting (Halmann *et al.* 1999). A product named Activate 2001®, containing *B. laterosporus*, *B. licheniformis* and *B. chitinosporus* decreased root-knot nematode (*M. incognita* and *M. arenaria*) populations when applied to 60 day old acerola plants (*Malpighia glabra* L.), an important crop fruit in Central America (Farias-Larios *et al.* 2000). *B. chitinosporus* also occurs as a contaminant and food-poisoning agent in pasteurized milk, UHT milk, cheese

(Cosentino *et al.* 1997), evaporated milk (Kaligridou & Vassiliadou 1992), milk based infant foods (Rowan *et al.* 1997), infant feed formula, vanilla sauce (Salkinoja-Salonen *et al.* 1999) and bread and meat dishes (Varnam & Evans 1991).

It would therefore be advisable to test withholding periods for Biostart 2000® to ensure consumer and environmental safety.

Furfural (2-furfuraldehyde) is a byproduct of the sugar industry and is a highly effective indirect nematicide that changes the soil microflora by stimulating bacteria antagonistic to nematodes and reducing their numbers (Zeitsch 2000). Furfural demonstrated control activity against *M. incognita* and fungal diseases on cotton (Bauske *et al.* 1997). It also reduced numbers of *Helicotylenchus dihystera*, *Paratrichodorus spp.*, *Pratylenchus zeae*, *Tylenchorhynchus sp.*, *Xiphinema elongatum* and *X. mampara* in the root zone of sugar cane (Spaull 1997). Furfural was effective in reducing preplant nematode populations on pineapple (Sipes *et al.* 1997). It also controlled *M. javanica* in the roots of cucumber and eggplant (Al Hamdany *et al.* 1999).

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CHAPTER 5
GENERAL CONCLUSIONS

Various aspects of woolly apple aphid, *Eriosoma lanigerum*, were investigated. This included initial galling damage caused by *E. lanigerum* to the roots of apple trees, the possible relationship between *E. lanigerum* and *Xiphinema* and *Pratylenchus* nematodes and the effects of Biostart 2000® and Furfural® as possible control agents of *E. lanigerum* in the orchard.

Preliminary root damage was characterized by the V-shaped mechanical rupturing of the epidermal and parenchyma tissue of the cortex as first instar *E. lanigerum* started feeding on the roots. The damage stretched to the vascular cylinder surrounded by the endodermis while torn parenchyma cell walls led to the increase of intercellular air spaces.

The damage caused by second, third and fourth instar woolly apple aphid was similar although the symptoms were more pronounced. Large amounts of cortex parenchyma were removed even from areas not adjacent to the entry point of the proboscis. This suggested that damage was caused by the chemical action of saliva as well as the mechanical penetration of the stylet. Growing root tissues caused the root to split lengthwise along the side already weakened by 3rd instar *E. lanigerum* feeding. The split stretched from the epidermis to the endodermis of the vascular bundle. Damage to the endodermis was apparent only after feeding by 4th instar *E. lanigerum*. Root tissues responded during this stage with the slight swelling of roots at infected areas suggesting the involvement of enzymatic damage.

Damage to the vascular bundle appeared only during feeding by adult *E. lanigerum*. The damage caused by adults included the noticeably increased swelling of the infected area of the root. Cell walls hardened until the whole root was radially

strengthened with reams of dead sclerenchyma cells and non-conducting xylem vessels while the cuticle grew to many times its original thickness through the growth of cork-like cambium tissues. Xylem and phloem formation was abnormal due to the tearing of the vascular rays and disjointed occurrence of secondary (non-conducting) xylem and phloem tissues. Apart from forming unsightly galls, these abnormalities in growth seriously impair the normal functioning of tissues, causing more stress under taxing environmental conditions and ultimately leads to an inferior crop.

There was no direct relationship between the population dynamics of *E. lanigerum* and those of *Xiphinema* and *Pratylenchus penetrans* nematodes. The occurrence of *E. lanigerum* appeared to be seasonal at both Molteno and Sandy, while there was no apparent pattern in *Xiphinema* numbers as they remained low for most of the study period, increasing suddenly towards the end of the study period at both sites. There also appeared to be a seasonal cycle in *E. lanigerum* numbers at Oak Valley while *P. penetrans* numbers fluctuated erratically at Oak Valley.

Root growth and root nitrogen levels seemed to correspond with the normal root growth cycle, peaking during spring and autumn. The nitrogen levels from galled roots were significantly lower than those of undamaged roots at both Molteno and Oak Valley. Only at Molteno were the nitrogen levels of galled roots significantly lower than those of roots taken from between galled areas. The consistently lower root nitrogen levels from galls were probably due to *E. lanigerum* feeding.

It is not certain whether different soil types influenced *E. lanigerum* and nematode population levels. Soils rich in fine sand and clay sustained higher populations of *E. lanigerum* and *Xiphinema* than sandy soils. Soils rich in clay have a greater amount of fine particles and a greater capacity to trap free water. Dry soil favoured *E. lanigerum*. Soils rich in clay also have a greater ability to expand and

contract, forming a series of cracks in the soil through which *E. lanigerum* nymphs and adults can move more freely.

The numbers of *E. lanigerum* found in soil samples correlated well with the damage index allocated to the samples. The numbers of *Xiphinema* found in soil samples also correlated well with the index allocated to the samples according to suspected *Xiphinema* damage symptoms. This suggests that the damage was probably caused by *Xiphinema* nematodes.

Both Biostart 2000® and Furfural® were effective as control agents of woolly apple aphid. Furfural®, a waste product of the sugarcane industry, was not as effective as Biostart 2000®, a product that includes an activator and three bacterial species, *Bacillus laterosporus*, *B. chitinosporus* and *B. licheniformis*.

A probable reason for the added effect of the Biostart 2000® was the multiplying capacity of the bacteria. The bacteria in the Biostart 2000® treated pots could replicate themselves under suitable conditions while Furfural® dilutes with each watering. Biostart 2000® is also much easier to prepare than Furfural® since the components of Biostart 2000® readily mix to form a paste easily thinned by water, whereas Furfural® is an oily substance that does not easily to form a suspension in water. Furfural®, being a byproduct of the sugarcane industry, was surprisingly, almost 6 times more expensive than Biostart 2000®. Biostart 2000® does seem to be a very effective control agent of *E. lanigerum* under favourable conditions.

Woolly apple aphid can be a damaging pest – especially when soil and orchard conditions are favourable for its development and although there was no observed relationship between *E. lanigerum* and *Xiphinema* and *Pratylenchus* nematodes in the root zone of apple trees, all three can seriously affect apple tree vigour. The population sizes and damage to roots can however be monitored and managed with

timely applications of chemicals such as Biostart 2000® and Furfural®, especially in combination with mulching and the use of resistant rootstocks.

Initial galling by *E. lanigerum* was mechanical and only by the stage that adult *E. lanigerum* were feeding, did the root respond by forming less permeable tissues. There was no apparent relationship between numbers of *E. lanigerum* and *Xiphinema* or *E. lanigerum* and *Pratylenchus* nematodes. Biostart 2000® was superior as a control agent of *E. lanigerum* to Furfural®.